BAT USE OF UPLAND PONDS WITHIN A HARDWOOD FOREST ECOSYSTEM, SOUTHERN INDIANA

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BY:

KATELYN P. HARRISON

DEPARTMENT OF BIOLOGY

ADVISOR: DR. TIMOTHY C. CARTER

BALL STATE UNIVERSITY
MUNCIE, INDIANA
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ABSTRACT

THESIS: Bat Use of Upland Ponds within a Hardwood Forest Ecosystem, Southern Indiana

STUDENT: Katelyn P. Harrison

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The extensive forested uplands of Morgan-Monroe and Yellowwood State Forests (MMYSF) in southern Indiana are largely composed of ephemeral streams. The Indiana Department of Natural Resources created ponds within the state forests to provide year-round fresh water for wildlife. The goal of this study was to document bat activity at these ponds, and determine what habitat attributes influence bat activity levels. In 2018 and 2019, we conducted acoustic surveys at 25 ponds, and temporally paired sites in the surrounding forest, during the summer months. Vegetation structure surveys were conducted at each pond to quantify habitat attributes that could affect bat activity, and aquatic faunal surveys were used as a surrogate for water quality. Results showed that bat activity was significantly higher at ponds than forest sites. A two-way ANOVA revealed that only two of 25 ponds surveyed had significantly more bat activity than the average, and results were consistent between years. According to a random forest model, the amount of vegetation surrounding ponds had the strongest relationship with bat activity compared to the other variables. Ponds with the highest bat activity had the following characteristics: < 35% vegetation structure surrounding the pond, surface area ≥ 1000 m^2, and surface area coverage with emergent vegetation between 40-60%. In 2019, a small treatment experiment was conducted on a subsample of three ponds that were the least active in 2018, and two random selected ponds, not previously surveyed. Four 11m x 2m flyways were created around each of the five ponds by removing midstory trees and shrubs, to test if lowering the amount of vegetation surrounding a pond would increase overall bat activity levels. We used a two-way ANOVA with repeated measure and observed no difference bat activity levels between acoustic surveys, suggesting that bat activity is influence by other habitat attributes, in addition to vegetation structure. However, there were multiple outside influences within this single year experiment that may have influenced our results.

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INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

North American bat species are classified under the suborder Microchiroptera, which is in part distinguished by the ability for laryngeal echolocation (Gunnell and Simmons 2005). The 17 extant bat families that make up this suborder are highly adaptive both behaviorally and morphologically (Barclay and Brigham 1991). Microchiroptera exploit a variety of food sources, roosting preferences, and migration patterns. Documenting these bats species on a spatial level can be difficult due to behavioral variability, and certain common traits cannot be generalized among bat families (Barclay 1999; Denzinger and Schnitzler 2013; Peixoto et al. 2018). However, acquiring a baseline understanding of bat biology is necessary for the health and management of the taxonomic group. Therefore, survey locations should be focused where bats are known to overlap, when developing effective research surveys for documenting spatial presence involving multiple bat species. For this reason, a considerable amount of bat research has occurred over riparian zones, river systems, and still bodies of water (Russo and Jones 2003; Brooks and Ford 2005; Amorim et al. 2018). Watershed systems provide four valuable bat habitat components: open foraging space, abundant prey, drinking water, and traveling corridors (Brooks and Ford 2005). Standard survey methods often prioritize these sites because water sources draw in individuals from surrounding populations giving a picture of presence at a localized level (Loeb and O'Keefe 2006; Comer et al. 2014).

There are seven bat species known to inhabit the hardwood forest ecosystem of southern Indiana (IDNR 2020; Indiana State University, unpublished data; Whitaker Jr et al. 2002). While ranges of some southeastern bat species include Indiana, my study will only consider those species that are annually documented returning to Morgan-Monroe and Yellowwood State forests (MMYSF) for the summer season. The bats of Indiana physically differ in size and wing

morphology (Indiana Department of Natural Resources 2018). These physical differences influence the foraging behavior and echolocation call characteristics (Norberg and Rayner 1987). Large-bodied bats are characterized by having a high wing to body mass ratio designed for fast flying and agility (Denzinger and Schnitzler 2013). Echolocation calls unique to large-bodied bats are characterized with low frequency, narrow band-width, and minimal attenuation (Fenton et al. 2012). In contrast, small-bodied bats are adapted to forage within denser vegetation. Their wing morphology is short and broad for slower flying and greater maneuverability. Small-bodied bats have adapted their echolocation calls to distinguish prey from vegetation by using either insect flutter detection or passive gleaning (Denzinger and Schnitzler 2013). Echolocation calls unique to small-bodied bats are characterized by high frequency and broad bandwidth (Barclay and Brigham 1991). The ability to identify bat species based on unique echolocation calls has allowed researchers to correlate bat activity with habitat use.

The watershed systems of MMYSF are composed primarily of ephemeral streams. Unlike most of the northern and central parts of Indiana's landscape that is characterized by relatively low topographic relief and poor draining soil, MMYSF has steep topography and porous soil (Logan et al. 1922; Natural Resource Conservation Service 2011). This combination of features is not conducive to maintaining flowing watersheds throughout the year. The Indiana Department of Natural Resources (IDNR), Division of Fish and Wildlife (DFW) were concerned with a water inconsistency for wildlife within MMYSF, and to mitigate, ponds were built within the forests boundaries to provide water throughout the year (IDNR 2020a). If bats are attracted to watershed systems, then it can be assumed that MMYSF bats are using the ponds for foraging, drinking, and travel, however, this assumption has not been tested.

The MMYSF are actively managed, employing both an uneven and even-aged silvicultural methods (INDR 2020b). There is a priority set by the Division of Forestry (DoF) to maintain compartment-level habitat features for the benefit of forest fauna, but particularly those listed as Indiana State Species of Special Concern. The DoF uses silviculture management guidelines to maintain or create these habitat features. These guidelines emphasize maintaining the habitat quality and structural integrity of the ponds throughout MMYSF and a 11 m buffer around water sources was allocated as a limited harvesting area (IDNR 2020a). Although these guidelines do not impede timber harvesting, it does minimize disturbances to roost/den trees, tree snags, downed logs, and maintains a minimum canopy cover. Differences have been observed among ponds regarding habitat and forest structure; however, it is unknown how these structural attributes contribute to the level of bat activity.

LITERATURE REVIEW

Echolocation

Echolocation is as synonymous with bats as flight. Echolocation is the process of projecting soundwaves into an environment and creating an auditory scene from the echoes of objects within that environment (Moss and Surlykke 2001). The mammalian order Chiroptera is hypothesized to have had a tandem evolution of laryngeal echolocation and flight approximately 85 to 65 million years ago (Springer et al. 2003). There is much debate regarding this hypothesis (Norberg and Rayner 1987; Eick et al. 2005; Teeling et al. 2005; Simmons et al. 2008; Veselka et al. 2010), but the morphological and molecular evidence supports the theory that laryngeal echolocation evolved once by a common ancestor (Simmons and geisler 1998; Springer et al. 2003). This common ancestor was likely an arboreal mammal that used primitive laryngeal

echolocation for orientation within its habitat (Simmons and geisler 1998). Fossil records suggest that early chiropterans were sight-based foragers, and laryngeal echolocation was limited to obstacle detection. Echolocation seen in extant bats was likely refined after wing morphology and cochlea structure evolved for detection and pursuit of aerial prey (Simmons and geisler 1998). All extant bat families, except Pteropodidae, use laryngeal echolocation (Fenton et al. 2012). Pteropodids are hypothesized to have lost the ability for laryngeal echolocation over time, but the *Rousettus* genus has been documented using tongue clicks (Griffin et al. 1958), showing a secondary gain of another type of echolocation. The remaining extant bats have retained laryngeal echolocation and variegated the ability to exploit different niches. This, coupled with the ability for flight, is the reason for the success of Chiropteran radiation (Teeling et al. 2005; Jones and Holderied 2007).

The utility of echolocation developed rapidly in the late Eocene period when ancestral bat species foraging style shifted from perch gleaners to aerial hawking (Simmons and geisler 1998). These early bat species are hypothesized to have used low duty cycle echolocation. A duty cycle is the ratio between the length of sound compared to the length of silence until the next sound is produce. Duty cycles are associated with the vocalization patterns that bats emit while foraging (Fenton et al. 2012). In general, low duty cycle bats will have high amounts of silence between each vocalization. This mechanic prevents the echoes of potential prey from being masked by the bat's own vocalizations (Kalko and Schnitzler 1989). Low duty cycle bats are often associated with frequency modulated vocalizations. These are "search calls" emitted during foraging that change over time, usually from high to low (Fenton 1995). Bats can adjust these search calls in both frequency and length when approaching and catching a target. High duty cycle echolocation is a second form that is hypothesized to have evolved from low duty cycle echolocation

(Simmons and geisler 1998; Fenton et al. 2012). High duty cycle echolocation is distinguished by the long duration of sound produced by a bat compared to the short duration of silence from each search call. These bats are still listening for prey, but vocalize at a different frequency than the target. Additionally, calls are often produced at a constant frequency or seen fluctuating less than 1 kHz. High duty cycle bats also use the Doppler-shifted echoes created by the bat moving around its target by lowering the frequency of their calls as distance from the target is reduced (Schnitzler 1973; Jen and Kamada 1982). High duty cycle echolocation is thought to be specialized for insectivorous bat species inhabiting high clutter areas because it is only found in old world Rhinolophidae, and Hipposideridae families, and one species of Mormoopidae, *Pternous parnellii*. In particular, the Doppler-shifted echoes of flutter insects provide bat species enough information to continually locate prey within dense vegetation (Bell and Fenton 1984).

Species-specific echolocation calls have been observed across bat families (Simmons et al. 1979; Fenton and Bell 1981). Unique echolocation structure is an adaptive behavior that is influenced by a species' morphological and environmental constraints within a habitat (Neuweiler 1983). These unique calls are observed almost entirely through foraging search calls (Barclay 1986; Obrist 1995). Bats produce echolocation calls to find available food sources and the auditory scene gathered is dependent on the returning echoes (Barclay and Brigham 1991). A prey target's returning echoes are dependent on size, thus bats must match the frequency range to find that target. In general, a species' call bandwidth and duration is correlated to the size of a prey target (Brigham 1990; Barclay and Brigham 1991). For example, small bat species produce high frequency search calls because low frequencies do not reflect small prey targets adequately (Barclay and Brigham 1991). Other evolutionary pressures for species specific search calls are atmospheric attenuation, background obstacles, and auditory camouflaged (Møhl 1988). The

unique search calls of different bat species is a reflection of those pressures, in addition to foraging style, wing morphology, and body mass (Aldridge and Rautenbach 1987). This is observed in the subtle differences of search calls between cohabitating species that share the same resources (Barclay 1999).

Echolocation allows bat species to exploit a variety of niches and food sources (Neuweiler 1990). The sound emitted may vary depending if a bat is searching for food or a roost site. Sound manipulation allows an individual to acquire an enormous amount of information about its surroundings. The components of an echolocation call are frequency range, bandwidth, duration, and harmonics (Jones and Teeling 2006; Jones and Holderied 2007). The expression of a call's structure is often associated with wing morphology (Aldridge and Rautenbach 1987). Wing shape and size dictates which prey bats are most suited to acquire (Freeman 1981), and echolocation call structure must be adapted to find those targets (Broders et al. 2004). For example, the adaptive variations of echolocation structures can be observed between two North American Vespertilionidae species, *Euderma maculatum* and *Eptesicus fuscus*. Both species are similar in size, foraging style, and can occupy the same forested habitat when foraging. Prey selection differs as *E. maculatum* primarily feeds on moths and *E. fuscus* is more of an insect generalist (Woodsworth et al. 1981; Leonard and Fenton 1984; Wai-Ping and Fenton 1989). The echolocation structure for *E. maculatum* is a low frequency range between 6-15 kHz, narrow bandwidth, and contains at least two harmonics. This structure allows *E. maculatum* to approach their main food sources, tympanic moths, without being detected (Woodsworth et al. 1981). Contrastingly, *E. fuscus* can produce calls with a broad bandwidth between 25-76 kHz (Griffin 1971; Bell and Fenton 1984), up to 8 ms in length (Fenton et al. 1983), and contains one harmonic (Bell 1980). *E. fuscus* has be recorded preying upon moths, beetles, flies, and shield

bugs (Whitaker Jr et al. 1977; Griffith and Gates 1985; Brigham 1990; Whitaker Jr 1995); hence, call structure must be flexible to find each type of insect. In fact, *E. fuscus* individuals have been observed adjusting the frequency range and duration depending on regionality (Brigham and Cebek 1989; Obrist 1989). The discovery of these unique call structures (Griffin 1958) has become a valuable tool for studying bats.

Acoustic Equipment and Detectors

The first recording apparatus used to hear a bat's ultrasonic call was a modified A.M. radio receiver with an audio output (Pierce and Griffin 1938). The radio was sensitive enough to demonstrate that little brown bats (*Myotis lucifugus*) produced rapid and constant echolocation pulses to avoid obstacles, giving insight to how bats orientate themselves in an environment (Griffin and Galambos 1941). Advances in microphone sensitivity allowed researchers to observe frequency ranges coinciding with specific species, and adding the use of an oscilloscope revealed the unique frequency sweep or "shape" of echolocation calls (Griffin 1946). However, microphone sensitivity has primarily been the main limitation in documenting a bat's echolocation call (Griffin 2004). Low intensity or "whispering bats" are often undocumented because of limitations in a microphone's recording range (Griffin 2004; Kalko 2004). Additionally, high frequency echolocation calls (100+ (kHz)) are more affected by atmospheric attenuation, shortening the distance that sound can travel (Griffin 1971; Lawrence and Simmons 1982). Most modern microphones combat atmospheric attenuation through preamplifiers, a broadband recording range, and omnidirectional capabilities for maximum coverage. Increasing the range and power of a microphone creates a trade-off between increased sensitive but a higher echolocation signal-to-noise ratio (Pettersson 2004), making it difficult to accurately identify

species. Most modern recorders compensate via manual or automatic setting adjustments that can prevent or reduce noise from overexposing a recording (Wildlife Acoustics 2020).

Bat detectors employ one of several methods to convert echolocation calls from ultrasonic to audible sounds. Each method has benefits and disadvantages that affect the probability of detecting a bat, and the amount of discernable information from a search call. A trained listener can document certain behaviors, frequency levels, and differences between species (Griffin 2004; Pettersson 2004).

Heterodyne detectors use a narrowband conversion method, in which the user can tune the recorder, usually at a width of 10 kHz, limiting the observe calls within a specific frequency (Pettersson 2004). For example, setting the recorder to 40 kHz will record all calls from the 45 kHz to 35 kHz range and filter out all other frequencies. Heterodyne detectors playback calls in real time. Tonal, repetitive, and durational differences can be heard between high and low frequency species groups, in addition to foraging behaviors like feeding buzzes (Griffin 2004; Pettersson 2004). The narrowband width increases sensitivity and lowers the signal to noise ratio. The drawback to this method is the inability to document bats outside of the frequency range settings, and potentially clipping calls from broad bandwidth bats that begin or end outside of the set frequency width (Pettersson 2004).

Frequency division detectors provide the broadband alternate to heterodyne detectors (Pettersson 2004). Calls across a microphones range are recorded continuously. Echolocation calls are made audible by reducing the sound frequencies to a fraction of the original, creating a square wave, also known as a zero-cross signal (Corben 2004; Pettersson 2004; Hourigan and Corben 2012). Frequency division detectors also playback calls in real time, and provide the same information as heterodyne detectors. However, broadband capabilities of frequency

division detectors create a high signal-to-noise ratio, confounding certain characteristics like harmonic presence (Corben 2004; Pettersson 2004; Szewczak 2004). Additionally, the zero-crossing transformation tends to lose some tonal and durational information about the signal, thus are not recommended for on-site species identification (Corben 2004; Pettersson 2004).

A time expansion detector is another broadband method that digitalizes bat calls and saves them within an attached storage system. Calls are then audibly replayed to the listener by slowing the speed of the call, usually by a fraction of one-tenth the original speed (Ahlén and Pettersson 1985; Pettersson 1985). This playback feature is especially useful for high frequency calls in which the user can hear the change in frequency over time (Szewczak 2004). However, there is a lag between recording and playback, which allows some individual bats to pass undetected (Pettersson 2004). Despite that limitation, time expansion recorders are considered to be the more suitable for acoustic surveys (Griffin 2004). Time expansion detectors do not distort sound waves during recordings and, when combined with sound analysis software, provide users with more diagnostic details than a zero-crossing method.

Full spectrum detectors record the complete sound wave of a bat's search call. Recordings retain the original shape and structure of the sound wave showing the important diagnostic features of a species search call. However, time expansion software is required to hear ultrasonic playbacks. Assuming that a high-quality recording was obtained, full spectrum can display the most accurate information regarding call duration and amplitude (Fenton et al. 2001). This translates to better species identification because users can see subtleties like power, shape, and frequency range (Fenton et al. 2001; Szewczak 2004). Zero-crossing acoustic recordings can also be displayed using sound analysis software; however, information regarding call duration and frequency range is limited. The zero-crossing method transforms sound waves, which causes

certain call features like harmonics and amplitude to be lost (Corben 2004). Additionally, the visual display of full spectrum calls provide users with improved information to discern between noise and an echolocation signal (Szewczak 2004).

Sound Analysis Software

Acoustic call processing is rarely feasible at a field site. Acoustic surveys often take place over a large landscape, continuously for an extended period. Adding to the challenge, modern detectors provide an external storage system so that a high volume of echolocation pulses can be collected. An echolocation pulse is one vocalization signal produced by an individual bat (Fenton and Bell 1981). A bat may produce more than 10 pulses per second (Brigham and Cebek 1989), generating a large volume of data in a single night. This volume of calls requires using sound analysis software or quantitative methods (Syas et al. 2014; Herr et al. 1997; Clement et al. 2014). Echolocation analysis software typically categorizes bat pulses by the following parameters: duration, sweep (frequency modulation), frequency maximum, frequency minimum, frequency mean, frequency slope, and tail duration (Herr et al. 1997; Britzke 2003; Clement et al. 2014). Sound analysis software automates the collection of these measurements, and provides the user with access to regional call libraries and a user-friendly interface, which may be more cost-effective. Thus, species identification through sound analysis software has become an increasingly popular research tool among biologists.

The structure of an echolocation pulse is intended to be the most effective and efficient means of acquiring information (Fenton 2004). It is expected that the pulse parameters of morphologically similar species will likely overlap. In addition, bats express a high degree of behavioral plasticity in their vocalizations (Obrist 1995; Barclay 1999). Diagnostic echolocation pulse parameters are necessary for accurately identifying unique variations in a bat's search call.

Reference libraries are the cataloged echolocation pulse parameters of a known species. The potential accuracy in a software's analysis is highly dependent on how these reference calls were gathered. High quality reference libraries must contain a large sample of calls in a variety of environments, and, ideally, consist of search calls of free-flying bats (Szewczak 2004). Sampling effort for reference calls usually requires the capture of an individual for positive species identification. Bats are recorded during the release and followed by using light tagging (Hovorka et al. 1996; Britzke 2003), flying tethered to a zip line (Szewczak 2000), or visual identification with a spotlight (Szewczak 2004). Each method requires a high degree of experience, manpower, money, and time to create a robust quality library. Another option is using public records or online call libraries. These public call libraries are often specialized in a specific region, and may only provide one or two reference calls per species (Erickson and West 2020). The quality of these online libraries is not always known. Ideally, an online call library should be transparent about how and where pulses were collected, such as the North American Bat Monitoring Program (NABAT) (Reichert et al. 2018). NABAT has established an online call library across multiregional and multinational borders. The goal is to establish standardized protocols for acoustic surveys to eliminate bias from survey discrepancies (Reichert et al. 2018). This program has a robust selection of reference calls across North America and the backing of many acoustic experts.

Historically, the Titley Scientific (Titley Electronics, Ballina, NSW, Australia) Anabat recorder and Echoclass zero-crossing analysis software dominated the North American bioacoustics market (O'Farrell et al. 1999; Duffy et al. 2000; Milne et al. 2003, 2004). However, there is now a lucrative market for creating ultrasonic analysis software as researchers began to see the potential positives in bioacoustics and the price reduction of data processing. Currently,

there are four North American specific analysis software programs available to users: Echoclass, Sonobat (Szewczak 2020), Bat Call Identification (BCID) (Ball Call Identification Kansas City, MO) and Wildlife Acoustics Kaleidoscope Pro (KPro; Wildlife Acoustics, Inc Maynard, MA). These programs advertise the ability to accurately identify recorded bat calls to species, but there has been little direct comparison among the programs (Tyburec 2015; Smith 2019). One comparison showed vastly different species classification numbers when all four programs were tested with the same manually vetted dataset (Tyburec 2015). This is likely due in part to the different reference libraries used by each manufacturer. This is particularly important to consider when assessing regional call variations within a species (Brigham and Cebek 1989). Only BCID has a limited analysis range to the eastern United States, and the other three programs advertise analysis of all bat species in North America. In general, software manufactures are aware of the limitations in acoustic analysis, especially for species that are considered hard to detect or overlap pulse parameters with other species (Szewczak 2004; Wildlife Acoustics 2019). Some manufactures provide workshops for users on best practice acoustic survey techniques and guides to manual species identification (Wildlife Acoustics 2019; Szewczak 2020).

Acoustic Surveys

Researchers have used simple ultrasonic recording equipment to identify species based on unique search calls (Bell and Fenton 1984). Fenton and Bell (1981) suggested that the change in frequency range, call duration, and shape, over time are the most diagnostic structures of a species' search call. Call structures are best observed through an oscillograph and, more recently a spectrogram, to see the visual representation of echolocation pulses. These tools allow researchers to discover more subtle diagnostic structures, like the appearance of acoustic harmonics and temporal intensity (Woodsworth et al. 1981). Acoustic surveys are used

internationally, and researchers have continued to catalog search calls under natural or semi-natural settings from bat species (Rydell et al. 2002; Walters et al. 2012; Taylor et al. 2013). Acoustic surveys can provide biologists with insights about bat behavior, ecology and evolution (Bell 1980; Kalko and Schnitzler 1989; Jones and Holderied 2007); however, this technique is just one available survey tool.

Passive acoustic surveys are common because they can be deployed quickly by one or two people, autonomously record for many months, and yield massive amounts of data. When compared to capture surveys, like mist netting, recorders are not confined to areas of known high use to gather data, such as riparian zones or traveling corridors. The equipment is not known to influence avoidance behavior, which is often observed from repetitive capture surveys at the same location (Marques et al. 2013). Akin to bird point counts, acoustic surveys provide information on bat presence in an area over time, and if surveys are conducted annually, biologists can interpret relative presence through activity trends (Ford et al. 2011; Ralph et al. 1995; Parsons et al. 2003). Acoustic surveys are especially advantageous for documenting bats living in open landscapes or flying at high altitude, such as *Euderma maculatum* and *Lasiurus cinereus,* which are difficult to capture, but are identified acoustically from their unique search calls (O'Farrell and Gannon 1999). Acoustic surveys can measure the volume of habitat use occurring by bat species across a landscape gradient. This question is often asked about threatened or endangered species in order to protect important habitat features from anthropogenic disturbances (Ford et al. 2005). Acoustic surveys are commonly used within forested ecosystems to answer questions of forest structure effects, silvicultural influences (Elmore et al. 2005; Yates and Muzika 2006), and in recent years , abundance decline due to the

impacts of white-nose syndrome (Ford et al. 2011) and wind energy farms (Rodhouse et al. 2019).

Acoustic surveys can provide large amounts of data, but there is a limit to the derived interpretations and conclusions. Confirming a species presence within a community should be approached cautiously. Bats produce search calls to acquire information about their surroundings and can adjust the frequency and shape with the environment. Call variations by individuals are documented during foraging bouts (Barclay 1986), regionality differences (Barclay 1999), and changes in habitat structure (Russo and Voigt 2016). Additionally, weather effects and habitat structure influences will impact the ability of microphones to detect a bat (Caldwell 2015; Neece et al. 2019). Recent software advances and available online reference libraries have created a user-friendly platform for autoclassification (e.g., Sonobat, KPro, Echoclass). Species identification should be confirmed with manual vetting, only after receiving adequate training. Capture surveys should be used in tandem to confirm presence, especially with extra-range detections, (Parkins et al. 2016) because acoustic surveys favor species that produce low frequency and high intensity calls (Griffin 1958). There is always potential that even long-term acoustic surveys can miss quieter species (Waters and Jones 1994).

Foraging Guilds

Clutter is the structural composition that bats must navigate while searching for and catching prey, and is the last component in determining habitat use (Fenton 1990). Clutter is usually defined as any non-prey object producing echoes (Schnitzler et al. 2003). Clutter generates two selective pressures within a habitat. A bat must be able to identify potential food sources within the clutter, and then mechanically navigate to, or chase, that food source through clutter without risking injury (Fenton 1990). The evolutionary response from these two pressures optimized

echolocation structure and wing morphology for different clutter levels. The sensory behavior and mechanical limitations define a bat's foraging structure or foraging group (Aldridge and Rautenbach 1987; Norberg and Rayner 1987). Foraging groups are separated into three degrees of clutter conditions from open (low), edge (mid) or narrow (high) space foragers (Aldridge and Rautenbach 1987; Norberg and Rayner 1987; Denzinger et al. 2004).

Open space foragers have optimized wing morphology and echolocation structure for no clutter obstacles and produce long-range, low frequency echolocation calls to identify prey over a large area. These foragers have a high aspect ratio and wing loading, and use an aerial hawking method to acquire prey (Schnitzler et al. 2003; Denzinger and Schnitzler 2013). Edge foragers must locate prey within a cluttered background and adjust flight pathing to avoid collisions. Edge foragers produce a wide and complicated range of echolocation calls that are adjusted depending on distance to the target. In general, call duration is kept short to avoid echo masking, but this reduces the call strength to identify prey. Edge foragers compensate by using shallowly modulated calls for prey identification upon approach, and then switch to steeply modulated calls for acquisition (Denzinger and Schnitzler 2013). The modulation switch can be observed acoustically by the unique presence of two feeding buzzes (Schnitzler 1987; Denzinger et al. 2001; Ratcliffe et al. 2011). Narrow space foragers that detected prey using active echolocation are primarily high duty cycle species. High duty cycle bats can identify the differences in echoes between unmodulated clutter and fluttering insects (Fenton et al. 2012). Other species forgo active echolocation and use olfactory or prey generated noise to locate targets (Schnitzler et al. 2003). Bats can exhibit a high degree of behavior plasticity within a foraging habitat. There is overlap in foraging location for clutter adapted species to hunt within a more open environment

(Schnitzler and Kalko 2001), thus placement within a group should be define as the most efficient means of foraging, rather than physical exclusion (Fenton 1990).

Measuring Activity

Habitat use is defined as the time and energy that an animal uses to acquire the resources that are available in a habitat (Krausman 1999). Understanding habitat use by bat species is difficult to visually observe because bats are nocturnal, highly mobile and, for most species, inaudible to human hearing. Direct methods are used to study certain aspects of habitat use (e.g., guano contents, roost tree selection (Whitaker Jr 1995; Menzel, M. A. et al. 2002)), but pose a logistic challenge for documenting bats across a large scale. Bat passes are a common measurement of bat activity, and often defined as the number of recorded sequences of >2 echolocation pulses from an individual, separated by one second (Fenton 1970). This method cannot distinguish the number of passes collected from individuals; therefore, we cannot estimate population sizes but can estimate relative bat activity over time (Kunz et al. 2007). Acoustic surveys can be used to measure relative bat activity within a habitat or compare activity across multiple habitats, using an Acoustic Activity Index. This measurement totals the number bat passes and divides them by the survey effort (time), creating a less biased conclusion on bat activity within a habitat in comparison to solely counting passes from individual bats (Miller 2001). In general, habitats with a higher Acoustic Activity Index indicate higher use, but survey repetition is necessary to interpret temporal changes in habitat use (Frick 2013).

Activity levels of bat species within a habitat are correlated to wing morphology and echolocation structure (Aldridge and Rautenbach 1987; Norberg and Rayner 1987). The mechanical limitations enforced by flight influences the size of a bat and, subsequently, its feeding strategy (Norberg and Rayner 1987). Flight has a high energy cost, especially for larger

bat species, and prey selection must compensate for energy use. Although the basic wing morphology is relatively the same among all bat species (Fenton 1995), there are differences in aspect ratio, wing loading, and wingtip shape that are consistent with certain foraging styles (Norberg and Rayner 1987). Foraging styles can be categorized into three main groups: hawking, gleaning/hovering, and trawling. There are additional foraging styles but, for this study, I will only be focusing on those used by temperate bat species (Norberg and Rayner 1987; Kalko and Schnitzler 1989).

Foraging Behavior

Hawking foragers chase down prey while airborne, like birds of prey. Fast hawking bats are distinguished by a high aspect ratio, high wing loading, and pointed wingtips. This allows bats to travel at high velocity but retain high maneuverability. Having a high aspect ratio also allows a lower cost to longer foraging sessions (Norberg and Rayner 1987). Echolocation structure for these foragers are commonly long, narrowband calls that are adapted to identify prey targets, but does not give good detail for background objects (Denzinger and Schnitzler 2013). In contrast, slow hawking bats have low aspect ratio, low wing loading, and rounded wing tips for maximum maneuverability (Norberg and Rayner 1987). These species are often associated with areas of high clutter, and their echolocation reflects that by primarily being longer calls at a constant frequency. These morphologic characteristics are most associated with high duty cycle bats (Fenton et al. 2012; Denzinger and Schnitzler 2013).

Gleaning or hovering foragers, on average, have a low aspect ratio, and wing loading for slow flying. Wing morphology for this foraging style can vary depending on the clutter within the habitat. Hover-gleaning bats will produce low intensity and short echolocation calls primarily for orientation, and texture discrimination (Norberg and Rayner 1987). Carnivorous gleaners

often do not use echolocation while foraging, and listen for sound made by targets along the ground (Fuzessery et al. 1993).

Trawling foragers are attracted to swarming insects over water or on the water's surface. Uniquely, these species often have high aspect ratios and low wing loading with pointed wings that allow for slow, maneuverable flying for long periods of time (Norberg and Rayner 1987). Echolocation structure is varied. Most bats using the trawling style produce both constant and frequency modulated calls as a search phase, but switch solely to frequency modulated when tracking prey (Norberg and Rayner 1987).

Objectives

The purpose of this study is to use acoustic surveys to document bat use at ponds within the MMYSF during the summer months. Bat use was estimated through activity levels via the number of acoustic files recorded at a pond during a three-night period, divided by the average hourly survey effort. Activity level was compared between all ponds to determine if use was equal across the MMYSF. Surrounding vegetation structure and water quality attributes of ponds were measured to gain a better understanding of what habitat attributes contribute to variation in activity levels at pond sites. If bat activity is influenced by local-scale features including average diameter at breast height (DBH) of trees >6cm in diameter and >4.5m tall, clutter cover, and canopy closure, then ponds with higher surrounding vegetation will have lower activity levels. Lower activity levels could be attributed to the exclusion of larger bat species. If removing the vegetation that surrounds a pond increases bat activity, then high cluttered ponds may not be suitable for larger bat species. Lastly, control sites not associated with ponds or other water sources were surveyed for bat activity in tandem with pond sites. My hypothesis is that bat

activity will be higher at pond sites because the ponds and the areas immediately surrounding them provide foraging locations, roosting habitat, and drinking water for summer bat colonies.

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CHAPTER 2

THE BEST PLACE TO GET A DRINK: BAT ACTIVITY AT UPLAND PONDS WITHIN A HARDWOOD FOREST ECOSYSTEM, SOUTHERN INDIANA.

Harrison, K.P., and T.C. Carter. To be submitted to Journal of Forest Science.

Abstract

The extensive forested uplands of Morgan-Monroe and Yellowwood State Forests (MMYSF) in southern Indiana are largely composed of ephemeral streams. The Indiana Department of Natural Resources (IDNR) created ponds within the state forests to provide year-round fresh water for wildlife. Previous MMYSF studies have supported the positive relationship between bat activity and these water resources. The goal of this study is to document bat activity at these ponds and determine what habitat attributes influence bat activity levels. We conducted summer acoustic surveys at 25 ponds, and temporally paired sites in the surrounding forest. Vegetation structure surveys were conducted at each pond to quantify habitat attributes that could affect bat use, and aquatic faunal surveys were used as a surrogate for water quality. Results indicated that bat activity was significantly greater at ponds than forest sites. A two-way ANOVA revealed that only two of 25 ponds surveyed had significantly more bat activity than the average, and results were consistent between years. All ponds contained aquatic fauna across both survey years and were considered usable by bats for drinking. According to a random forest model, the amount of vegetation structure surrounding ponds had the strongest relationship with bat activity compared to the other variables. Ponds with the highest bat activity had the following characteristics: < 35% vegetation structure coverage surrounding the pond, surface area ≥ 1000 m^2, and surface area coverage with emergent vegetation between 40-60%. When making considerations for habitat improvement, land managers are correct in prioritizing vegetation structure; however, our results conclude bat activity is also dependent on pond size and hydrophilic plant productivity.

Keywords
Acoustic surveys, aquatic resources, bats, forest structure, habitat use

1.0 Introduction

Before European colonization, the Indiana landscape consisted of 19 million acres of continuous hardwood forests (Opie 1998; Martin et al. 2008). By the 1900s, homesteaders had removed nearly all of Indiana's hardwood forests for agriculture, domestic use, and construction, leaving only 1.4 million acres intact (Parker 1989; MacCleery 1993; Jenkins 2013). Indiana is a highly productive agricultural state that has ~15 million acres dedicated to farming (Indiana State Department of Agriculture 2020). The average farm landscape in Indiana is a monoculture for the production of corn, soybeans, and livestock. Forested habitat is often fragmented among these farms, but can be found throughout riparian zones, as nature preserves, or privately owned woodlots (Purcell et al. 2014; Red-Tail Land Conservancy 2020). Present-day hardwood forest habitats have seen some recovery. As of 2010, five million acres, or 21%, of Indiana has recovered some aspect of hardwood forested habitat. About 85% of the available five million acres of hardwood forests in Indiana are privately owned (Divison of Forestry 2010). Land use goals on these properties may not always provide suitable wildlife habitat. Fragmentation of viable habitat for wildlife is detrimental to populations, especially for migratory species (Robinson et al. 1995; Collinge 1996; Carman 2013), who depend on the limited rest sites available for foraging and replenishing fat stores (Packett and Dunning 2009). There is inevitably more pressure on the remaining 750,000 acres of public forests to have habitat that can support a range of wildlife species.

The Morgan-Monroe and Yellowwood State Forest (MMYSF) complex contains two adjoining properties in the south-central portion of Indiana that provide essential hardwood forest habitat to a variety of wildlife species. This region of Indiana is home to many federally and state-listed bat species (Indiana Department of Natural Resources 2018). The Indiana

Department of Natural Resources (IDNR), Division of Forestry (DoF) uses various silvicultural techniques to protect and preserve important habitat features for bats. These techniques focus on protecting available roost trees and the limited available water sources within both forests. The MMYSF watershed is largely composed of ephemeral streams that are frequently dry in the summer. From 1965 to 1993, IDNR, Division of Fish and Wildlife (DWF) constructed small ponds to provide water access to wildlife year-round. Previous studies have documented high habitat use by bats near water (Power et al. 2004). Within the MMYSF, these ponds provide the closest accessible drinking water without bats flying long distances to forests' boundaries. Although the DoF has protected and documented bat presence at these ponds, there has not been a formal survey to measure bat activity. Subsequently, if bats are drawn to pond sites, it is unknown what habitat attributes influence bat activity levels.

The purpose of this study was to: 1) document bat activity levels at ponds within the MMYSF using acoustic surveys, 2) quantify and analyze aquatic and vegetation attributes that may influence bat activity at pond sites, and 3) establish an estimate of bat activity at a fixed distance from each pond to compare bat use between aquatic locations to non-aquatic locations. Based on previous research conducted within MMYSF, we predict that bat activity will be higher at pond locations when compared to non-aquatic sites (Power et al. 2004). Efforts from mist-netting captures suggest that bat activity will vary among ponds due to differences in size, surrounding vegetation structure, and water quality (Timothy C. Carter, [Ball State University, Muncie, IN], personal communication, May 1, 2020); Yates and Muzika 2006). We predict that vegetation structure around the ponds will have the highest influence on bat activity levels at pond sites (Loeb and O'Keefe 2006; O'Keefe et al. 2013).

2.0 Materials and Methods

2.1 Study Area

This study took place within the MMYSF, which is located within the counties Morgan, Monroe, and Brown of south-central Indiana. Both state forests are classified by the DoF as dominated by Mixed Upland Hardwood and Oak-Hickory forest types. The MMYSF complex is ~19,425 ha of continuous forest and makes up 30% of Indiana's state forests. Both forests are actively managed with even- and uneven-aged harvest methods (IDNR 2019). The MMYSF complex provides the public with excellent outdoor recreation, so the state puts a heavy emphasis for maintaining forest aesthetics. The landscape surrounding MMYSF is a combination of urban and agriculture. The agriculture fields are largely found in flat, previously bottomland habitat, and are prone to flooding. Unique to southern Indiana, the area has steep topography (Logan et al. 1922; Natural Resource Conservation Service 2011), and upland streams that are dry from May-August. MMYSF ponds were commonly constructed using dugouts along ridgelines.

2.2 Site Selection

We selected 25 of the ~73 MMYSF ponds in a systematic fashion so that they were distributed as evenly as possible across both forests; 11 in Yellowwood Forest, 10 in Morgan-Monroe Forest, and four centrally located along the border of both forests (Figure 1). Pond selection was prioritized based on vehicular accessibility; all ponds selected were within 100 m of a DoF road. Other pond attributes were unknown prior to initial surveys.

2.3 Acoustic Surveys

Passive acoustic surveys were used to monitor bat activity at ponds. We chose passive

surveys over capture surveys so that bat avoidance behavior did not bias pond activity (Larsen et al. 2007; Marques et al. 2013). Sites were surveyed in a south to north direction in 2018, and north to south in 2019 to account for temporal activity shifts from volant juveniles. Ponds were acoustically surveyed from the 21 May to 22 July in 2018 and 2019. Each pond was surveyed three times during the summer in three-week intervals, with each survey bout occurring over three consecutive nights, yielding a total of nine nights of bat activity data each year. We simultaneously deployed a "forest" recorder for each pond deployment to compare activity between pond and non-aquatic forested habitat. Forest recorders were placed 100 m from the pond's edge in a random cardinal direction. Cardinal directions for forest recording sites were not repeated for each subsequent survey. Each survey deployment consisted of either a Wildlife Acoustics SM2BAT or SM4BAT recording unit and corresponding omnidirectional microphone (Wildlife Acoustics, Inc., Maynard, MA). A majority of our acoustic recorders were SM2BATs; however, an effort was made to ensure that each pond had one survey with SM4BAT recorders. Microphones were placed on an improvised, 6 m high microphone stand that was made of interlocking polyvinyl chloride (PVC) pipes and secured at the end of a horizontal, 1 m long wooden dowel rod to avoid acoustic distortion. Microphone stands were secured in an open space with minimal clutter to maximize call quality. Microphone stands at pond sites were placed 1-3 m from the pond's edge to minimize acoustic echo from the water's surface. The acoustic units were set to record sound files in full spectrum and in a .WAV format. Units recorded from dusk until dawn, and were triggered to record for five seconds when sensing sound frequencies within the suggested factory default range for bats in Indiana (gain: 36 dB, dig HPF: fs/12, dig LPF: Off, trigger level: 18 SNR, trigger win: 2.0 s, div ratio: 16).

2.4 Vegetation Structure

The vegetation structure surrounding each pond was quantified at each pond's initial acoustic survey for both years. Vegetation structure was measured along four 11 m x 2 m transects, radiating from the pond's edge in each cardinal direction. Vegetation structure attributes were classified as canopy closure, pond canopy closure, clutter cover, and average diameter at breast height (DBH). DBH was measured at trees $\geq$ 6 cm in diameter and $\geq$ 4.5 m tall along each transect. Canopy closure was visually estimated into a percentile category: 0-25%, 26-50%, 51-75%, 76-100%. Clutter cover was defined as the amount of collective vegetation (tree, branches, shrubs) between a bat and the pond along the 11 m x 2 m transect. Clutter cover was visually estimated in a cone from the 11 m mark on the transect, and vertically up to a height of 17 m within the subcanopy. Clutter cover was estimated into a percentile category: 0-25%, 26-50%, 51-75%, 76-100%. Averages of canopy closure and clutter cover were converted to the percentile category midpoint. We also estimated canopy closure over the pond's surface using the previously stated methods. The visual presence of dead, free-standing trees (snags) $\geq$ 6 cm in diameter and $\geq$4.5 m tall was noted at each transect. Lastly, the percent cover of emergent vegetation in each pond was estimated as the complement of open water divided by surface area multiplied by 100.

Vegetation structure was compared between pond and forest sites using two 11 m x 2 m transects, centering from the recording location. Canopy closure and clutter cover at forest sites were estimated into a percentile category: 0-25%, 26-50%, 51-75%, 76-100% and then converted to the percentile midpoint. Survey time was limited during the field season, so we surveyed 50 of the forest sites in 2018 and the remaining 50 in 2019.

2.5 Aquatic Surveys

We used aquatic faunal presence as a surrogate for water quality to test our hypothesis that each pond could be a source of drinking water for bats within the MMYSF. Aquatic faunal surveys were conducted three times for each year and coincided with each acoustic survey deployment. If faunal presence was confirmed at a pond at any time during a summer survey season, we deemed it usable by bats for drinking water. Aquatic faunal presence was sampled via dipnet and visual inspection. Visual presence surveys continued throughout the acoustic deployment setup and stopped once an individual was spotted. One dipnet survey consisted of two single sweeps, 15 m apart, to access invertebrates underneath leaf litter (Fellers and Freel 1995). The number of macroinvertebrates within a net were counted and categorized to the lowest taxonomic hierarchy determined by unaided visual inspection. Vertebrates were categorized to type and life stage (e.g., adult frog), but not identified to species. Water temperature was recorded twice at the edge of the pond before each dipnet sweep. Lastly, at the initial acoustic survey, the diameter of each pond was measured using GPS waypoints for later surface area calculations.

2.6 Acoustic Classification

Recorded ultrasonic files were processed through Wildlife Acoustics' Kaleidoscope Pro autoclassification software (Wildlife Acoustics 2019). Our species list was chosen from summer mist netting records after 2011, when white-nose syndrome was first detected (Indiana State University (ISU), unpublished data). Little brown bat (*Myotis lucifugus*) was removed from our species list due to the low capture records post white-nose syndrome presence. We used the Kaleidoscope Pro V5.1.9 autoclassifier library customized for big brown bat (*Eptesicus fuscus*), eastern red bat, (*Lasiurus borealis*), hoary bat (*Lasiurus cinereus*), silver-haired bat, (*Lasionycteris noctivagans*), northern long-eared bat, (*Myotis septentrionalis*), Indiana bat

(*Myotis sodalis*), and the tri-colored bat, (*Perimyotis subflavus*). Kaleidoscope Pro detection parameters for echolocation pulse frequency were set between 130 kHz and 15 kHz, pulse duration 2 ms, and inter-syllable gap set between 500-2 ms were set to factory default. A pond's relative activity level was expressed as the number of sound files containing positive bat passes divided by survey effort. A bat pass was confirmed from a sound file that contained at least two pulses (Fenton 1970). If multiple bats were present in one sound file, then the file would only count as an activity index of one. Species were identified solely through Kaleidoscope Pro autoclassification software. We manually vetted low quality acoustic files to minimize data lost from over filtering by the autoclassification software. Positive confirmation of a bat presence in low-quality acoustic files was used for activity analysis and were not identified to species. We summed files identified to species with manually vetted calls for each pond to compare relative activity levels among pond sites.

2.7 Bat Activity and Pond Attribute Analyses

We used a two sample t-test [Wilcox-Signed Rank] to compare differences in forest and pond canopy closure and clutter cover. Changes in pond vegetation structure between years were compared using a paired t-test. Data were log transformed to meet t-test assumptions when applicable, or compared using a non-parametric t-test.

Our primary goal for this study was to document bat activity among ponds within MMYSF using the compiled acoustic activity from all species. Relative bat activity for forest and pond acoustic surveys were calculated by dividing average number of bat passes per each three night survey by the average number of survey hours (9.2 hrs). A two sample two-tailed t-test was used to compare the mean relative activity between forest and pond sites. A two-way ANOVA was used to compare relative bat activity between pond sites, survey year, and a

combination of pond site and survey year. We eliminated the influence of the surrounding forest habitat on pond activity by calculating the difference in bat passes between ponds and its temporally paired forest sites. Our acoustic data required a log transformation to meet the ANOVA normality assumptions. We used a post-hoc multiple comparison test to identify significant activity level differences between ponds. We used a random forest model regression to relate the variables of vegetation structure (average DBH, canopy closure, clutter cover, snag presence) and pond attributes (pond canopy closure, emergent vegetation cover, surface area) to relative bat activity. We used a correlation test to compare vegetation structure between average DBH, canopy closure, and clutter cover, to reduce redundancy within our model. We did not include aquatic faunal presence or temperature as a predictor in our model because all ponds met the requirements of water quality for both years. Two pond attributes (mtry = 2) were compared at each tree node (n tree = 500) to calculate the node purity value (IncNodePurity). Pond attributes with higher node purity will have a higher influence on predicting bat activity levels at a pond.

3.0 Results

3.1 Acoustic activity

There was a total of 105,519 .wav files containing bat passes for the 2018 and 2019 survey season. Over 75% of the total bat passes recorded occurred at pond sites. In 2018, we recorded 36,480 bat passes at pond sites and 10,721 bat passes at forest survey sites. In the 2019, we recorded 40,154 bat passes at ponds sites and 18,164 at forest survey sites. Pond sites had significantly more activity than the temporally paired forest sites ($n = 25$, $p = 0.001$, $d = 0.84$ [95CI 0.45, 1.23]). After accounting for survey effort and subtracting forest activity from each

corresponding pond, our 10 northern ponds averaged the highest activity among the 25 surveyed and the four center ponds averaged the lowest (Figure 1).

3.2 Vegetation Structure

Vegetation structure at forest sites averaged 55% (CI ± 4.8%) from canopy closure and 40% (± 6.27%) for clutter cover. A nonparametric t-test showed no difference detected between forest and pond sites regarding canopy closure (n = 25, p = 0.12, d = 0.42 [-2.5, 4.53e^{-5}]) and clutter cover attributes (n = 25, p = 0.11, d = 0.47 [-3.48e^{-5}, 2.5]).

Vegetation structure surrounding the ponds was recorded at all sites for both years. We observed a lower average clutter cover within the surrounding forest from 49% (± 9.4%) in 2018 to 35% (± 5.8%) in 2019 (n = 25, p = 0.01, d = 13.82 [2.85, 24.8]). Canopy closure within the surrounding forest averaged 47% (± 7.4%) in 2018 and 44% (± 4.7%) in 2019 and no difference was detected between years (n = 25, p = 0.24, d = 3.33 [-2.4, 9.06]). Average canopy closure among ponds was higher between years, from 32% (± 5.1%) in 2018, to 57% (± 7%) in 2019 (n = 25, p = 0.0002, d = -37.7 [-50, -25]). Average DBH increased from 19 cm (± 2) to 43 cm (± 6.7) between years (n = 25, p = 0.0001, d = -0.8 [-1.01, -0.58]). Average emergent vegetation cover significantly increased from 22% (± 9.4%) to 36% (± 10.6%) between years (n = 25, p = 0.0001, d = -0.77 [-1.21, -0.33]). Snag presence was documented among the vegetation surrounding 19 ponds in 2018 and all 25 ponds in 2019.

3.3 Aquatic results

We completed three aquatic faunal presence and dipnet surveys at 23 ponds in 2018, and were unable to complete initial aquatic presence surveys at two ponds (n = 73). Visual presence and dipnet surveys were successfully completed three times at each pond in 2019 (n = 75). Adult frogs in both survey years were the most observed amphibian life stage during visual presence

surveys (Appendix C). Macroinvertebrate visual presence was documented at 63% of ponds in 2018 and 76% in 2019, with Gerridae being the most common macroinvertebrate group recorded. Over three surveys we counted a total of 373 individuals in 2018 and 596 individuals in 2019 (Appendix C). Macroinvertebrates comprised 79% of total individuals in 2018 and 45% in 2019. The most frequent macroinvertebrate group recorded was Unknown in 2018, and Odonata in 2019. We observed a higher frequency and diversity of herpetofauna captures between years (Appendix C).

The surface area across 25 ponds averaged 363 m^2 (± 191) in 2018 and 187 m^2 (± 54) in 2019. Temperature recorded across all three dipnet surveys averaged 22˚C (± 1.2) in 2018 and 20˚C (± 1.2) in 2019.

3.4 Pond Attribute Analysis

Our two-way ANOVA analysis comparing activity levels among ponds showed that some ponds were significantly more active than others ($n = 25$, $p < 0.0001$, $f = 3.023$). A post-hoc multiple comparison showed that one pond was significantly more active than 11 of the 25 ponds that were surveyed, and a different pond was significantly more active than one of the 25 that were surveyed. No difference was detected between survey years ($n = 2$, $p = 0.9$, $f = 0.018$), or the site:year interaction ($n = 25$, $p = 0.8$, $f = 0.702$) (Appendix B).

We observed a high correlation between vegetation structure variables, canopy closure and, clutter cover ($r = 0.74$, $p < 0.0001$). Rather than omitting a variable, we averaged canopy closure and clutter cover into one variable: surrounding vegetation structure. According to the random forest model the hierarchy of attribute influence on bat activity was (in descending order): surrounding vegetation structure, pond surface area, emergent vegetation, average DBH, pond canopy cover, and snag presence ($v^2 = 0.14$) (Figure 2).

According to the Random Forest model, average bat activity tended to be highest at pond sites with <35% coverage of surrounding vegetation and ≥1000 m^2 surface area (Figure 3). Bat activity was higher at ponds with emergent vegetation covering 40-60% of the surface and were lowest with <20% coverage. Bat activity responded negatively at ponds with average DBH >45 cm and >20% pond canopy closure. Snag presence showed no clear positive or negative influence over bat activity levels. No significant difference was detected in our post hoc correlation comparison between pond canopy and emergent vegetation ($p = 0.13$) or surrounding vegetation structure and emergent vegetation ($p = 0.64$). Our data suggest that there is a negative relationship between pond canopy and increasing surface area ($p = 0.07$).

4.0 Discussion

Consistent with other studies, we found that ponds within the MMYSF are areas of high bat activity in comparison to only forested habitat (Ford et al. 2006; Nolder 2016; Titus 2018). Pond sites consistently averaged higher activity levels than forest sites for both survey years. Our data support the hypothesis that bats are attracted to aquatic habitats because they provide locations with food and water (Krusic et al. 1996). In addition to available foraging opportunities, all pond sites were within 100 m of DoF service roads. Roads provide edge habitat that many species within the MMYSF are known to travel and forage along (Grindal and Brigham 1999). There were a few insistences when a forest site would have higher activity levels than the corresponding pond survey. This may have been caused by a random survey location placing detectors adjacent to a DoF service road. We found no difference in vegetation structure between forest and pond sites; however, there were other vegetation variables that we did not consider for this study, such as a distinguishing between midstory and understory tree count. Our method of

visually estimating clutter can be inconsistent between surveyors and accurate distinction between our clutter percentile categories will vary by individual. O'Keefe et al. (2014) found that quantifying clutter by individual variables, such as subcanopy stem count and stem volume, is more effective at measuring clutter than visually estimated percentile categories. We defined clutter as the amount of vegetation between a bat and the pond along the 11 m transect; however, if we separated clutter into subcategories, such as subcanopy stem volume and understory stem volume, we could have a better understanding between the differences in our bat detection at forest and pond sites.

Our second hypothesis was that vegetation structure surrounding the ponds would have the greatest influence on bat activity. We found that the percent cover within the surrounding vegetation structure was the most important predictive variable. Our findings are in agreement with similar studies using habitat characteristics as predictors for bat activity. Loeb and O'Keefe (2006, 2011), concluded that bat activity decreases as the amount of forest vegetation increased around the recorder. A possible explanation is the exclusion of larger bat species due to their wing morphology and echolocation structure specialized for areas of low clutter (Brooks and Ford 2005; Bender et al. 2015). Another explanation for these results is the higher likelihood of bats being detected in low-clutter environments. Common protocol for acoustic surveys is to place recorders in low-clutter areas to decrease interference from the surrounding habitat. We did not analyze the effect of surrounding vegetation on bat detectability, but past studies in the MMYSF have concluded that habitats with less vegetative structure had higher use among the majority of MMYSF bat species (Nolder 2016; Titus 2018; Caldwell 2019).

Surface area was the second ranked attribute of importance to predicting bat activity at ponds. Our predictive plots show a positive trend in bat activity as pond surface area increases to

~1000 m^2. This trend was observed geographically: northern ponds were most active for both survey years and, on average, 300 and 200 m^2 larger than southern ponds and more centrally located ponds, respectively. For bats roosting within the MMYSF boundaries, these ponds are the most accessible drinking resource without traveling long distances to larger bodies of water. The bats of MMYSF only drink while in flight, so larger ponds provide more opportunity to approach and acquire water, which is especially beneficial for larger, less maneuverable bat species. An experiment by Hall et al. (2016), showed that when the surface area of a water source was reduced, larger species of bats had a significant decrease in successful drinking attempts. Larger bat species unable to use small ponds may be draw toward the northern state forest, Morgan-Monroe, or travel longer distances to preferred drinking sites outside of MMYSF. Additionally, a larger pond will likely have a lower canopy closure over the surface area, which could be important for bat species that travel above the canopy during foraging bouts (Kalcounis et al. 1999). Our study suggested that pond canopy and surface area were negatively correlated, but more information is needed. Something that our study did not record but could add a layer of explanation to our results is the relationship between pond shape and bat activity levels. A majority of ponds within MMYSF are round, and anecdotal observations have noted *L. borealis* individuals approaching these locations is tight spiral patterns to access drinking water. The two oblong ponds in our study were also among the most active. It could be worth comparing bat behavior between ponds of different shapes to provide an explanation for type of use (i.e. foraging vs drinking).

Contrary to our forest structure results, ponds with more emergent vegetation covering the surface water had higher average bat activity. This result could be related to the overall productivity of the aquatic ecosystem within the ponds. Hydrophilic plants are an important food

source, breeding location, and refuge for amphibians and, aquatic macroinvertebrate species (Okonkwo 2011). If productivity as an indicator of habitat quality, it could be argued that locations with more emergent vegetation offered more abundant foraging opportunities in addition to drinking water (De Szalay and Resh 2000). Although lower vegetation density and canopy cover allows more sunlight to reach aquatic vascular plants, our post hoc correlation comparison showed no relationship between pond canopy, surrounding vegetative structure, and emergent vegetation. We observed northern ponds having higher average activity levels overall, but the least amount of emergent vegetation among all three geographic survey locations. This supports our finding that surface area and surrounding vegetation structure are more important to predicting bat activity at MMYSF ponds.

There were few notable discrepancies within our results and survey locations between years. DBH at ponds significantly changed from 19 cm (95CI± 2) to 43 cm (± 6.7). It is doubtful that the surrounding vegetation had significant growth between years and most likely caused by a change in transect orientation. There were two pond sites altered by forest management between survey seasons. One pond had an adjacent DoF service road expanded in anticipation of a timber harvest. This caused the north and west portion of the pond's vegetation structure to be removed. Additionally, forest was removed to create a staging area less than 30 m from the pond's edge. There was an increase in bat passes by 119% per survey from 2018 to 2019. We would expect an increase in bat activity at this location due to the decrease in clutter and expansion of edge habitat, but one year of survey data is not enough to determine if vegetation removal was the cause. The second pond site was altered by a prescribed burn to encourage oak regeneration. Average bat passes were reduced by 43% per survey from 2018 to 2019. Canopy from mature trees were largely intact, but there was a noticeable decrease in clutter at the pond and forest

sites. Bat activity is relatively unaffected by prescribed burns (Lacki et al. 2017); however, insect populations are reduced after an initial burn creating less foraging opportunities for bats at that site (Silvis et al. 2016).

We recorded aquatic faunal presence at all ponds through a combination of visual and dipnet surveys for both years, and considered each pond to be useable by bats for at least drinking water. The presence of aquatic fauna in our study was only used as a surrogate for water quality because amphibians are known indicators for ecosystem health (Welsh and Ollivier 1998). Water sources are areas of high foraging activity for bats due to the large concentrations of available prey (Fukui et al. 2006). The macroinvertebrate taxonomic groups that we documented could be attractive to bats because a few studies have shown larger species like *E. fuscus* and *L. borealis* consume Coleoptera species (Brigham 1990; Whitaker Jr 2004). However, our goal for this aquatic survey was to document diurnal presence, and it is unknown what invertebrates were present nocturnally. Measuring both aquatic and terrestrial insect density at these ponds could also provide more insight into the relationship between bat activity and ponds overtime. Insect density at a location varies seasonally (Fukui et al. 2006), which could explain variation in bat activity levels between each survey repetition.

Our study found that ponds within MMYSF are areas of high bat activity and aquatic faunal presence. Ponds had significantly more activity than their surrounding forest habitat, suggesting that these locations are important habitat features for bat species. Our results can be used as justification for pond maintenance and recommendations of potential pond improvement to increase bat activity. Habitat attributes such as surrounding vegetation structure, surface area, and emergent vegetation appear to be important to predicting the level of bat activity at a pond. We found that ponds with the highest activity had <35% cover within the surrounding

vegetation, a surface area of ≥1000 m^2, and surface area coverage of 40-60% emergent vegetation. Future research that documents the proportional use between foraging and drinking by bats at these ponds will further contribute to understanding the effects of pond shape on use, and help to understand the effect of future habitat modifications. MMYSF ponds provide breeding habitat, foraging, and refuge locations for bats and other taxa, and are examples of the DoF's mission to promote high faunal diversity by providing and maintaining high quality wildlife habitat on state forest property.

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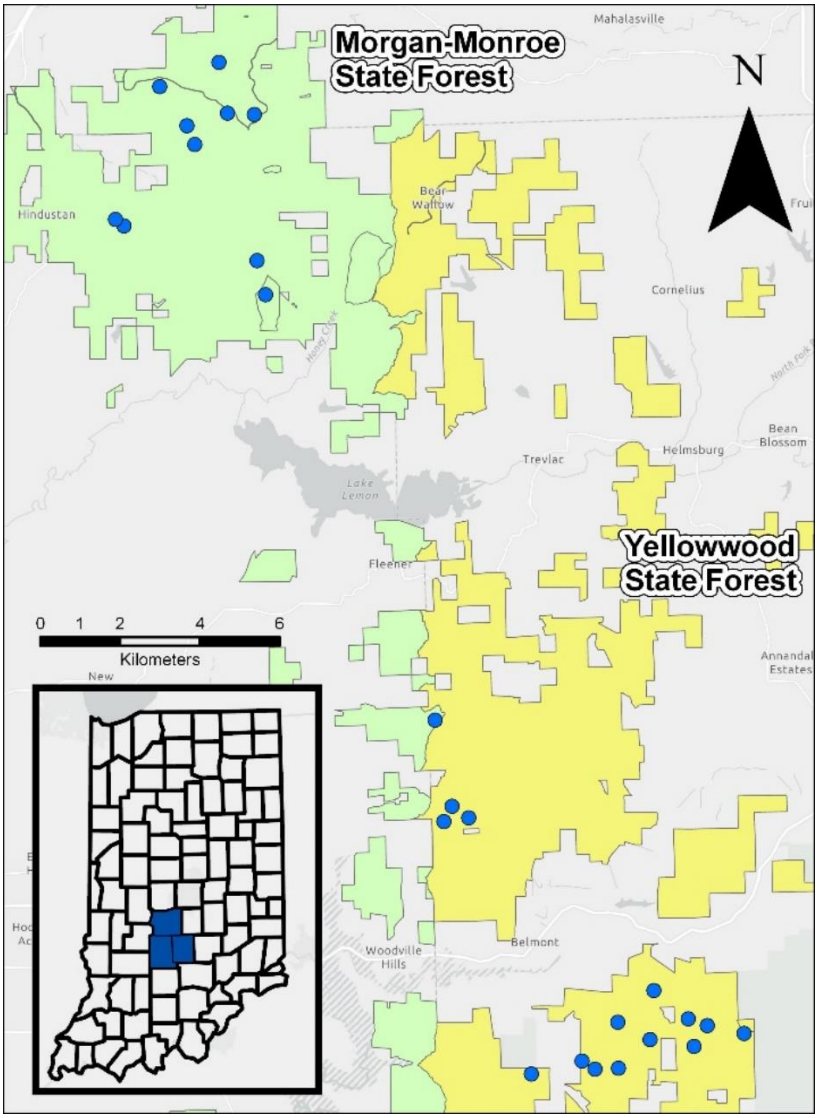

Figure 1. Map of the study area within the boundaries of the Morgan-Monroe and Yellowwood State Forests complex located in south-central Indiana, USA, May-July, 2018-2019. Acoustically surveyed wildlife ponds are represented as blue circles (n = 25).

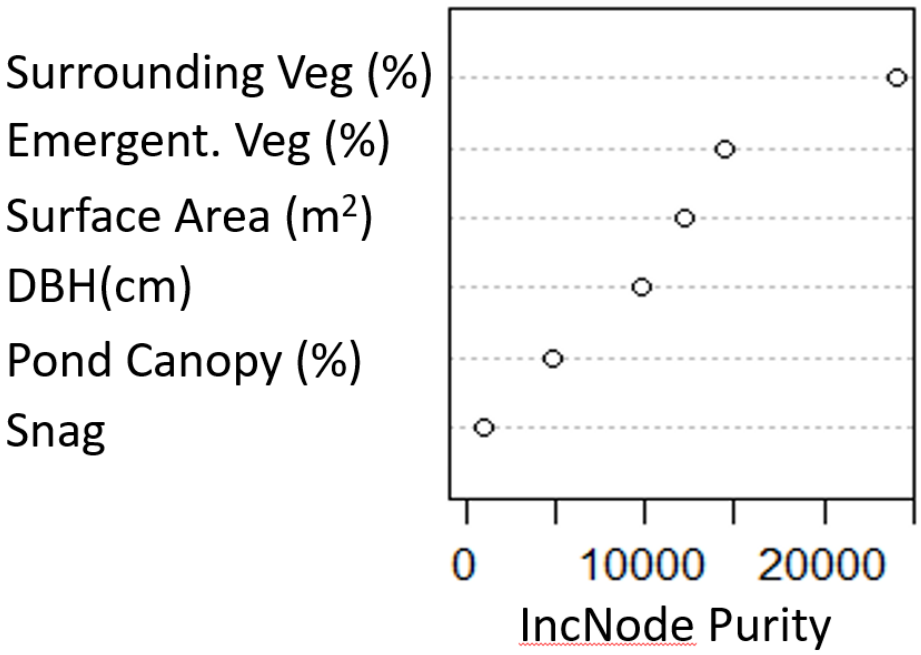

Figure 2. Random Forest results of pond attributes collected in 2018 and 2019 from MMYSF in southern Indiana. Pond attributes are ranked by highest influence on relative bat activity levels using node purity as the importance index.

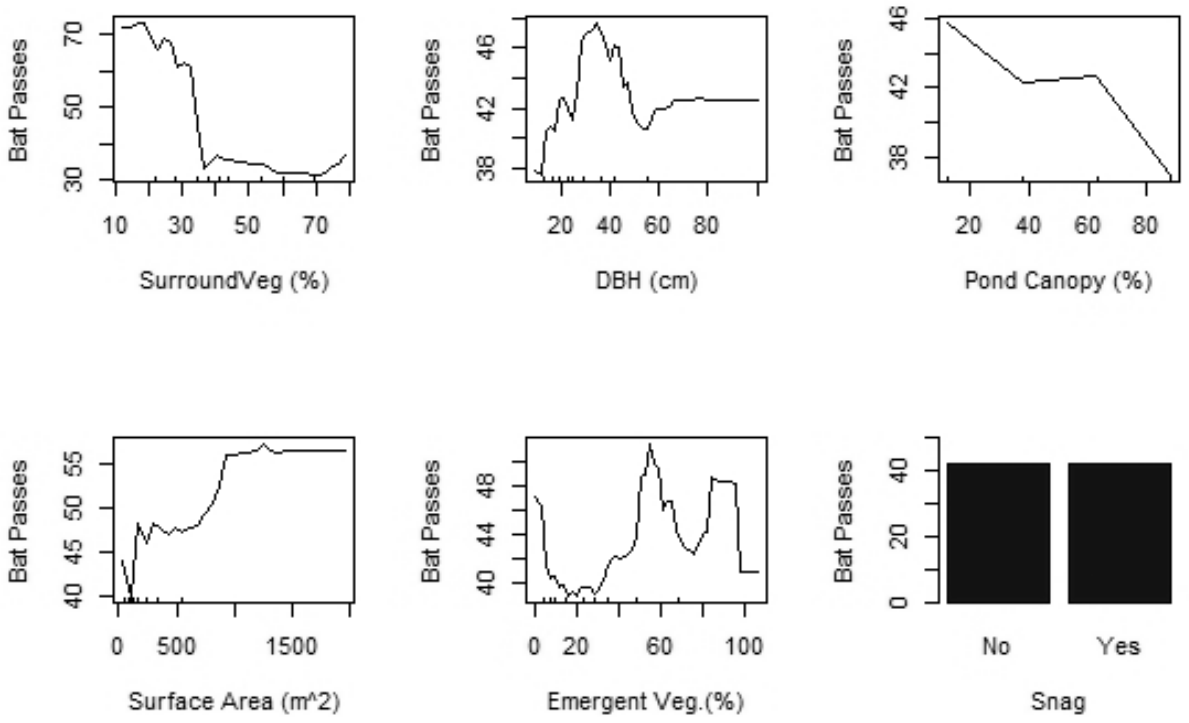

Figure 3. Predictive dependence plots depicting the range of effect that pond attributes have on relative bat activity during the 2018 and 2019 survey seasons within MMYSF of southern Indiana.

Appendix A.

Species four letter code:

EPFU= *Eptesicus fuscus*

LABO= *Lasiurus borealis*

LACI= *Lasiurus cinereus*

LANO= *Lasionycteris noctivagans*

MYSE= *Myotis Septentrionalis*

MYSO= *Myotis sodalis*

PESU= *Perimyotis subflavus*

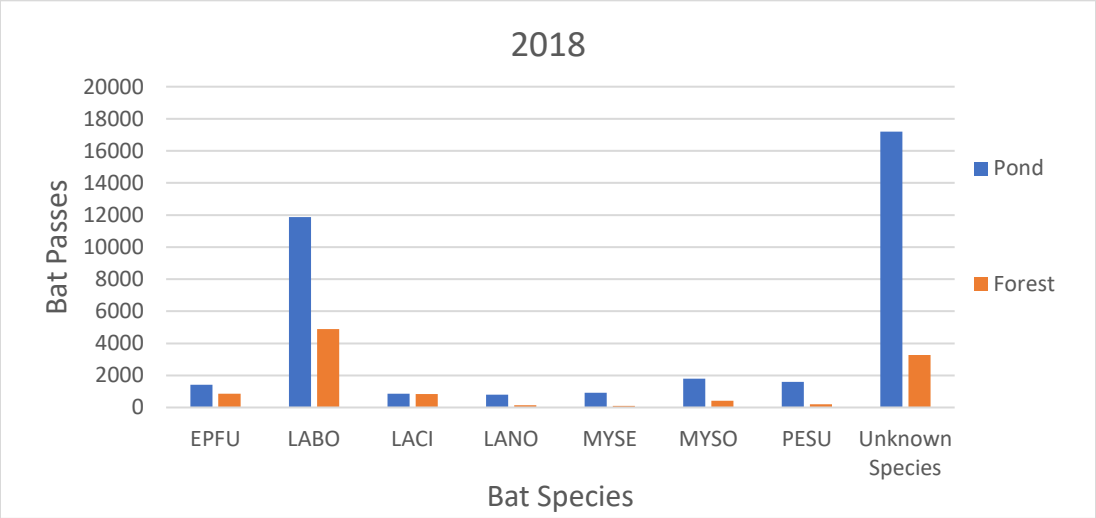

Figure 1. The total number of bat passes autoclassified to species by Wildlife Acoustics Kaleidoscope Pro software and manual vetted low-quality calls designated as Unknown Species for 2018. LABO (*L. borealis*) was the highest identified species recorded at wildlife ponds and forest sites. The highest quantity of recordings were manually vetted low-quality sound files.

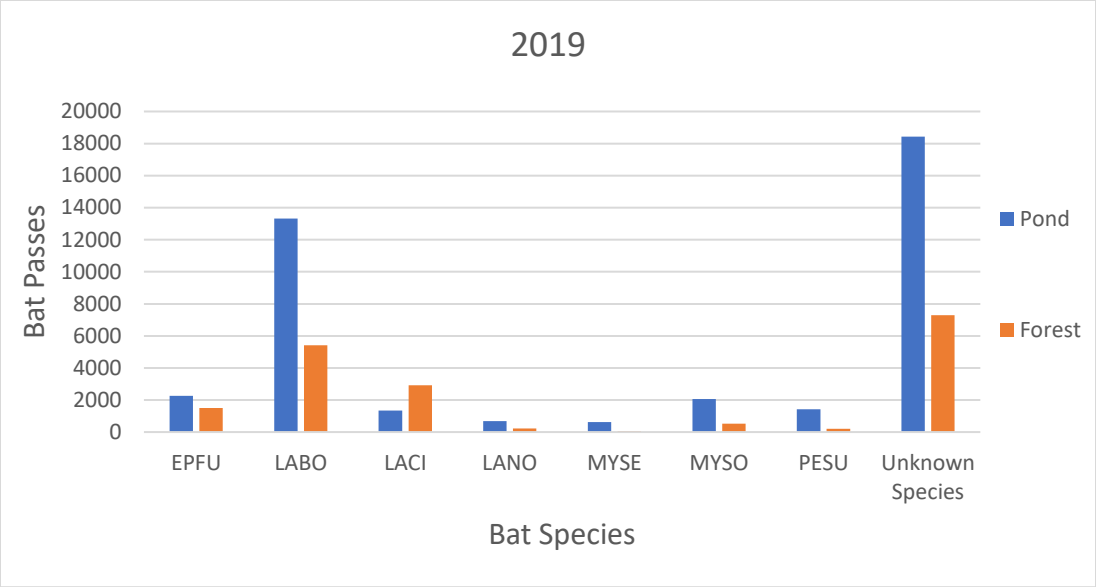

Figure 2. The total number of bat passes autoclassified to species by Wildlife Acoustics Kaleidoscope Pro software and manual vetted low-quality calls designated as Unknown Species for 2019. LABO (*L. borealis*) was the highest identified species recorded at wildlife ponds and forest sites. The highest quantity of recordings were manually vetted low-quality sound files.

Appendix B.

Table 1. Results from the two-way ANOVA summary table analyzing the activity level differences between wildlife pond (site) and survey year (year). Activity levels were determined from bat passes collected during the 2018 and 2019 survey season in Morgan-Monroe and Yellowwood State Forests in south-central Indiana.

ANOVA Variables	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	24	2.63	0.10957	3.023	6.05e-05 ***
Year	1	0.001	0.00064	0.018	0.894
Site: Year	24	0.61	0.02543	0.702	0.839
Residuals	100	3.624	0.03624		

Appendix C.

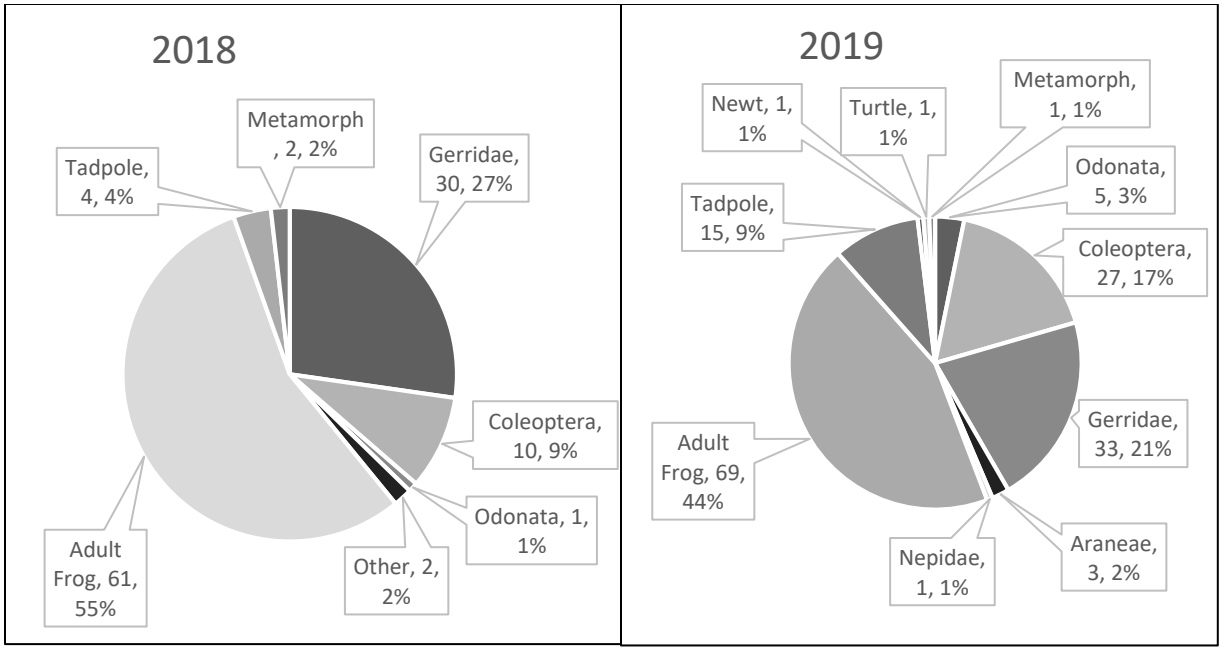

Figure 1. Visual presence survey results of aquatic fauna from 23 ponds in 2018 and 25 ponds in 2019 at MMYSF in southern Indiana. Ponds were surveyed three times in three week intervals over a nine week survey season.

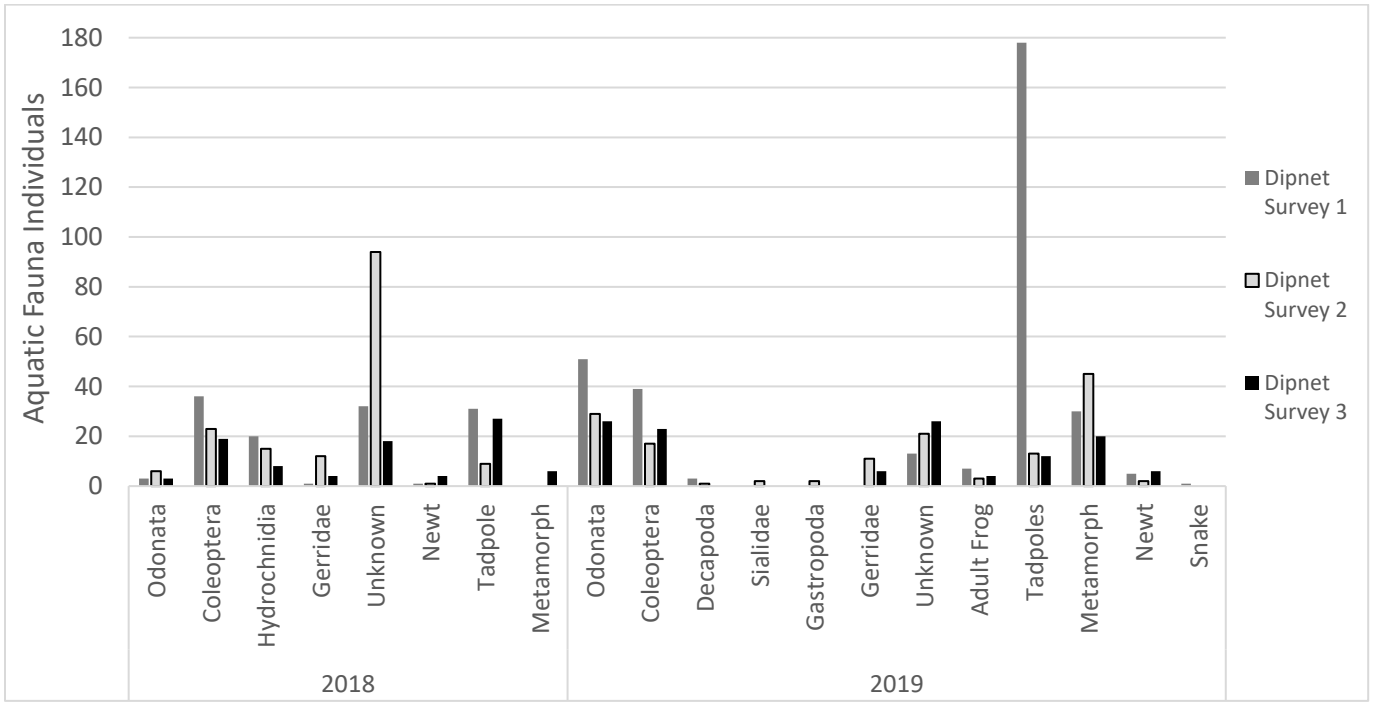

Figure 2. Mean number of aquatic fauna by type in dipnet samples collected from 23 ponds in 2018 and 25 ponds in 2019 at MMYSF in southern Indiana. Ponds were surveyed three times in three week intervals over a nine week survey season.

CHAPTER 3

BAT SHAMING: IS CLUTTER EXCLUDING LARGE-BODIED BATS FROM PONDS WITHIN A SOUTHERN INDIANA HARDWOOD FOREST ECOSYSTEM?

Harrison, K.P. and T.C. Carter. To be submitted to Journal of Forest Science.

ABSTRACT

Large-bodied bats are distinguished by long, narrow wings that are built for fast, sustained flight and low maneuverability. These species are associated with open habitats, and are often excluded from areas of high clutter or risk of colliding with background objects. Acoustic surveys were conducted at 25 ponds within Morgan-Monroe and Yellowwood State Forests (MMYSF) in southern Indiana in summer 2018 to determine bat activity levels for the season. Vegetation cover and bat activity were negatively correlated ($r = -0.76$, $p = 0.003$). Low activity at these ponds could be attributed to large-bodied bat exclusion due to high clutter within the midstory surrounding ponds. A small treatment experiment was conducted in 2019 on of three of the ponds that were the least active in 2018, and two random selected ponds that were not previously surveyed. We tested the hypothesis that low vegetation surrounding a pond will increase overall bat activity levels by removing midstory trees and shrubs to create four, 11 m x 2 m flyways radiating out from each of the five ponds. A two-way ANOVA with repeated measure found no difference in bat activity levels between acoustic surveys, suggesting that bat activity is influence by other habitat attributes in addition to clutter. However, there were multiple outside influences within this single year experiment that may have influenced our results. Further surveys are needed before using our results to support management decisions.

KEYWORDS

Acoustic surveys, bat activity, Chiroptera, forest management, treatment experiment

INTRODUCTION

The evolution of echolocation and flight has allowed bats to exploit a diversity of food sources within a habitat. Specialized wing morphology and echolocation frequency can predict how and where a bat will forage within a habitat (Denzinger and Schnitzler 2013). Open-space foragers are generally large-bodied bats with long, narrow wings that are built for fast flying and low maneuverability. These species have echolocation calls that are characterized by a low frequency range, narrow bandwidth, and slow attenuation (Fenton et al. 2012; Denzinger and Schnitzler 2013). Narrow-space foragers are usually small-bodied bats with short, wide wings that are designed for slower flight speeds but higher maneuverability (Denzinger and Schnitzler 2013). Echolocation calls for small-bodied bats have a high frequency range, large bandwidth and steep attenuation, allowing calls to be more resistant to clutter interference (Aldridge and Rautenbach 1987).

The amount of clutter within a habitat acts as an ecological filter. Clutter can create a barrier that forces some bat species to forage at locations that are better suited to their wing morphology and echolocation characteristics (Loeb and O'Keefe 2006; Ober and Hayes 2008; Adams et al. 2009). Large-bodied bats must avoid high-clutter habitats, or risk collision with background objects (Schnitzler et al. 2003). The foraging locations of forest-dwelling bats are directly influenced by the ecological filter of clutter (Obrist 1995; Loeb and O'Keefe 2006, 2011). Multiple studies have explored the relationship between vegetation clutter and habitat-use by bat species within forest stands and across forested landscapes. On average, large-bodied bat species are associated with reduced canopy closure such as clear cuts (Brooks and Ford 2005; Bender et al. 2015) and small-bodied bats are associated with the forest interior (Morris et al. 2010).

Morgan-Monroe and Yellowwood State Forests (MMYSF) of southern Indiana provide important summer roosting and maternity habitat for migratory and year-round residents. Southern Indiana has been white-nose syndrome positive since 2010, affecting small-bodied bat populations disproportionally to large-bodied bats (Silvis et al. 2016). Due to the spread of white-nose syndrome throughout the Midwest region, the most abundant bats in MMYSF are the large-bodied species (Caldwell 2015; Pettit and O'Keefe 2017; Titus 2018)**.** This study will focus on the seven bat species that have been captured within MMYSF after the arrival of white-nose syndrome (Indiana State University (ISU), unpublished data**)**. Four of these species are considered large-bodied: hoary bat, (*Lasiurus cinereus*), big brown bat, (*Eptesicus fuscus*), eastern red bat, (*Lasiurus borealis*), and silver-haired bat, (*Lasionycteris noctivagans*) (Indiana Department of Natural Resources 2018). The three small-bodied bats include the federally endangered Indiana bat, (*Myotis sodalis*), the federally threatened, northern long-eared bat, (*Myotis septentrionalis*), and the tri-colored bat, (*Perimyotis subflavus*) (Indiana Department of Natural Resources 2018; USFWS 2019). Reduced competition from the historically more abundance small-bodied bats can mean more flexible niche partitioning between species that allows large-bodied bats to occupy new areas within a hardwood forest (Jachowski et al. 2014).

The Indiana Department of Natural Resources (IDNR), Division of Fish and Wildlife (DWF) constructed small ponds within the MMYSF from 1965 to 1993 to provide wildlife with year-round access to water. Previous studies have documented high habitat use by bats near water (Power et al. 2004). Ponds within the MMYSF provide the closest accessible drinking water without bats flying long distances to forests boundaries. The IDNR, Division of Forestry (DoF) uses habitat management guidelines that maintain these important habitat features for wildlife, while promoting the growth of valuable commercial trees (IDNR 2019). Harvesting is limited to

<25% reduction in canopy and midstory around ponds, creating a variable amount of vegetation density between each aquatic location (IDNR 2020a). Ponds with higher vegetation density within the midstory clutter and canopy closure could exclude the large-bodied bats from drinking and foraging.

The objective of this study was to test if ponds with high midstory clutter and canopy closure exclude large-bodied bats from aquatic sites. Evidence from our 2018 survey year suggests a negative correlation between acoustic bat activity and vegetation structure at ponds ($r = -0.76$, $p = 0.003$; Chapter 2). Previous acoustic studies within MMYSF have also suggested that the activity of large-bodied bats tends to decline in areas with more clutter (Nolder 2016; Titus 2018)**.** We hypothesize that low activity levels are related to high clutter, and that reducing clutter around a pond, will increase activity levels.

STUDY AREA

This experiment took place within MMYSF located across the counties Morgan, Monroe, and Brown of south-central Indiana. The climate of southern Indiana is temperate, average summer temperatures range from 17℃ to 29℃ and average summer precipitation is 33 cm (NOAA 2020).

Both state forests are classified by the DoF as dominated by Mixed Upland Hardwood and Oak-Hickory forest types. The MMYSF complex is ~19,425 hectares of continuous forest, and makes up 30% of Indiana's state forests. Both forests are actively managed with even- and uneven-aged harvest methods (IDNR 2019). The MMYSF complex provides the public with excellent outdoor recreation, so the state puts a heavy emphasis for maintaining forest aesthetics. The landscape surrounding MMYSF is a combination of urban and agriculture. The agriculture

fields are largely found in flat, previously bottomland habitat and are prone to flooding. Unique to southern Indiana, the area has steep topography (Logan et al. 1922; Natural Resource Conservation Service 2011) and upland streams that are dry from May-August. MMYSF ponds were commonly constructed as circular dugouts along ridgelines.

METHODS

Site Selection

The ponds that were used in this experiment were a part of a larger study to determine relative bat activity levels at ponds within the MMYSF (Chapter 2). Ponds selected for this study were within 100 m from a DoF road, and continuously contained water throughout the survey season. Three treatment ponds were randomly selected from six with the lowest quantity of acoustic files recorded from our 2018 surveys (Chapter 2). Two additional treatment ponds that were not surveyed in 2018 were selected with the same requirements stated above (n = 5) (Figure 1). Prior knowledge of condition and attributes of the new treatment ponds were not known until the initial acoustic survey. Control ponds were selected using the remaining three ponds that had the lowest bat activity in 2018, and two randomly selected ponds from the 2018 survey season (n = 5).

Acoustic Surveys

Passive acoustic surveys were used to monitor bat activity at ponds in 2019 between May 21 and July 22. Each pond was surveyed three times during the summer in three-week intervals, with each survey bout occurring over three consecutive nights, yielding a total of nine nights of survey data for the season. If equipment malfunction was detected, we resurveyed ponds over the next consecutive three nights with new equipment. Each survey deployment consisted of either a

Wildlife Acoustics SM2BAT or SM4BAT recording unit and corresponding omnidirectional microphone (Wildlife Acoustics, Inc., Maynard, MA). A majority of our available acoustic recorders were SM2BATs; however, an effort was made to ensure that each pond had one survey with SM4BAT recorder. Microphones were placed on an improvised, 6 m high mic stand that was made of interlocking polyvinyl chloride (PVC) pipes and secured at the end of a horizontal, 1 m long wooden dowel rod to avoid acoustic distortion. Microphone stands were secured in an open space with minimal clutter, and placed 1-3 m from the pond surface to avoid an acoustic echo. The acoustic units were set to record in full spectrum from dusk to dawn, and save sound files in a .WAV format. Units were triggered to record for five seconds when sensing sound frequencies within the suggested factory default range for bats in Indiana (see Acoustic Classification) (gain: 36 dB, dig HPF: fs/12, dig LPF: Off, trigger level: 18 SNR, trigger win: 2.0 s, div ratio: 16).

Vegetation Structure Surveys

The vegetation structure surrounding each pond was quantified during the first survey of both years. Vegetation structure was measured along four 11 m x 2 m transects that radiated from the pond's edge in each cardinal direction. Attributes for vegetation structure were classified as canopy closure, clutter cover, and average diameter at breast height (DBH). DBH was measured at trees $\geq$6 cm in diameter and $\geq$4.5 m tall along each transect. Canopy closure was visually categorized as a percentile: 0-25%, 26-50%, 51-75%, 76-100%. Clutter cover was define as the amount of collective vegetation (tree, branches, shrubs) between a bat and the pond along the 11 m x 2 m transect. Clutter cover was visually estimated in a cone from a distance of 11 m from the pond, and vertically up to a height of 17 m within the subcanopy. Clutter cover was visually categorized as a percentile: 0-25%, 26-50%, 51-75%, 76-100%. Averages of canopy

closure and clutter cover were converted to the percentile category midpoint. We also estimated canopy closure over the pond using the previously stated methods. The visual presence of dead, free-standing trees (snags) ≥ 6 cm in diameter and ≥4.5 m tall was noted at each transect. Lastly, the percent cover of emergent vegetation in each pond was estimated as the complement of open water divided by surface area multiplied by 100.

Understory and midstory vegetation was thinned along the 11 m x 2 m transects at treatment ponds after completing the initial acoustic survey. Trees and shrubs <12.7 cm in diameter were removed manually with chainsaws and tree pruning shears. Vegetation over-hanging the water around the entire edge of the pond was also removed using the same methods. Vegetation remained intact at control ponds.

Aquatic Survey

Aquatic faunal surveys were used a surrogate for water quality testing and done three times coinciding with each acoustic survey deployment. If faunal presence was confirmed at a pond at any time throughout the survey season, we deemed it usable by bats for drinking water. Aquatic faunal presence was sampled via dipnet and visual inspection. Visual presence surveys continued throughout the acoustic deployment setup, and stopped once an individual was spotted. Lastly, the diameter of each pond was measured during the initial acoustic survey via GPS waypoints that were later used in surface area calculations.

Acoustic Classification

Recorded ultrasonic files were processed through Wildlife Acoustics' Kaleidoscope Pro autoclassification software (Wildlife Acoustics 2019). Our species list was chosen from summer mist netting records after 2011, when white-nose syndrome was first detected (Indiana State University, unpublished data). Little brown bat (*Myotis lucifugus*) was removed from our species

list due to the low capture records post white-nose syndrome presence. We used the Kaleidoscope Pro V5.1.9 autoclassifier library customized for big brown bat (*Eptesicus fuscus*), eastern red bat, (*Lasiurus borealis*), hoary bat (*Lasiurus cinereus*), silver-haired bat, (*Lasionycteris noctivagans*), northern long-eared bat, (*Myotis septentrionalis*), Indiana bat (*Myotis sodalis*), and the tri-colored bat, (*Perimyotis subflavus*). Kaleidoscope Pro detection parameters for echolocation pulse frequency were set between 130 kHz and 15 kHz, pulse duration 2 ms, and inter-syllable gap set between 500-2 ms were set to factory default. The relative activity level at a pond was expressed as the number of sound files containing positive bat passes divided by survey effort (avg. 9.2 hours). A bat pass was confirmed from a sound file that contained at least two pulses (Fenton 1970).

Activity Analysis

Relative bat activity at treatment and control ponds was calculated by dividing the number of bat passes for each three night survey by the average number of survey hours (9.2 hours). We used two-way ANOVA with repeated measures to compare differences in bat activity between each three night survey session. We collected vegetation structure data for treatment ponds prior to vegetation removal, and used a nonparametric, two-sample t-test to compared differences in vegetation structure between treatment and control ponds. Aquatic attributes for surface area, pond canopy closure, emergent vegetation of treatment and control ponds were also compared using a nonparametric two-sample t-test.

RESULTS

Pond Attributes

Aquatic fauna were present at all ponds and each acoustic deployment, so we considered all ponds to be sources of drinking water to MMYSF bats. A post-hoc analysis showed high correlation between vegetation structure variables, canopy closure and clutter cover ($r = 0.74$, $p < 0.0001$). Rather than omitting either variable, we averaged canopy closure and clutter cover into one variable: surrounding vegetation structure. No difference was detected between average coverage of the surrounding vegetation (Treatment = 55.5% (95CI±6%), Control = 63% (±13%), $p = 0.5$), average DBH (Treatment = 42 cm (±20), Control = 54.6 cm (±15.3), $p = 0.4$), average surface area (Treatment = 290 m^2 (±12), Control = 353 m^2 (±140), $p = 0.8$), average pond canopy closure (Treatment = 37.8% (±27%), Control = 27.8% (±12.2%), $p = 0.8$), average emergent vegetation (Treatment = 20% (±19.5%), Control = 55% (±15.7%), $p = 0.07$). A minimum of one snag was recorded at all ponds.

Activity Analysis

Our nine-week survey of treatment and control ponds produced 10,296 .wav files. Kaleidoscope software was able to identify 2,815 and 2,203 .wav files to a species for treatment and control ponds, respectively. From the files identified to species, large-bodied bat species comprised 75% of files at treatment ponds and 68% of files at control. Small-bodied bats comprised 25% at treatment ponds and 32% at control ponds. After accounting for survey effort (9.2 hours) the average, overall activity from both species groups was 20 bat passes/hour (±28) for treatment ponds and 16 bat passes/hour (±17) for control ponds. We observed no difference in bat activity between treatment and control ponds ($n = 15$, $p = 0.75$, $d = 1$ [95CI -16, 14]). According to our repeated measures, two-way ANOVA, there was no difference in bat activity

between each survey sessions at treatment ponds ($n = 3$, $p = 0.4$, $f = 1.04$) and control ponds ($n = 3$, $p = 0.7$, $f = 2.13$).

DISCUSSION

Removing a portion of the vegetation surrounding ponds at MMYSF did not result in increasing bat activity levels, despite control and treatment ponds having no significant differences in pond canopy closure, surface area, and emergent vegetation coverage. This experiment was unable to examine all of the complexities of aquatic habitats and hardwood forest ecosystem interactions with bat activity; however, some anecdotal and numerical data provide further insight.

As predicted, large-bodied bats were the most common species recorded within MMYSF and accounted for 75% of activity from all surveyed ponds. Similar to other MMYSF studies, our results documented higher activity from large-bodied bat species in comparison to small-bodied bat species (Caldwell 2015; Titus 2018). It is possible that the 5,278 .wav files that were not identified to species contained passes of small-bodied bats because high-frequency calls are often more difficult to obtain (Britzke et al. 2013), especially at control ponds where clutter was intact. There is also a recording bias for low-frequency sound waves because they travel further than higher frequency sound waves (Neuweiler 1990). These biases toward large-bodied bat calls prevented us from comparing activity levels between body size groups at ponds. Additional bias was observed from the difference in recording potential of our SM4 and SM2 units. A post hoc analysis revealed the recording potential of the SM4 units had approximately three times more acoustic files recorded per survey than the SM2 recorders. Therefore, ponds that were surveyed with our SM2 recorders likely underestimated bat activity. Repeated annual use likely meant that

the recording potential varied among SM2 units but equipment restraints prevented us from using the same recorder for each subsequent survey.

Unlike Loeb and O'Keefe 2006, in our experiment bat activity was not influenced by changes in vegetation structure at pond sites. We did not detect a difference in vegetation or aquatic attributes between treatment and control ponds, however, we had a small sample size, and attribute variation was high among ponds. Based on activity data from the previous year's surveys, ponds with low activity in 2018 remained with low activity through 2019 (Figure 2 and 3), suggesting that bats used these ponds less than other ponds for reasons besides high coverage within the surrounding vegetation. Notable attribute differences could explain why one or two ponds had higher or lower activity within a group. All ponds within this study had a small surface area based on data reported in Chapter 2. Within that study, ponds with a surface area averaging >400 m^2 had higher activity levels in comparison to smaller ponds. Nine of 10 ponds in this experiment were <400 m^2, and the only pond above 400 m^2 was control pond 2 (Figure 3), which had the highest activity among all control ponds. A single pond exhibited the predicted positive linear trend that we expected at the treatment locations (Figure 1). Treatment pond 1 (Figure 2) was randomly selected for this experiment, and not previously surveyed during the 2018 pilot year. It is difficult to conclude why this pond contained more bat activity despite having similar attributes to other treatment ponds. This pond was the only treatment pond to contain >50% emergent vegetation cover. Higher coverage of hydrophilic plants creates more refuge for macroinvertebrates, which creates a better foraging location than other treatment ponds used in this experiment (De Szalay and Resh 2000; Okonkwo 2011). One of our treatment ponds had a consistent negative trend in bat activity. This treatment pond had a reduction in the available water throughout the survey season, with more leaf litter and surface debris present by

the end of July. All bat species occurring in the MMYSF drink while in flight, and were likely unable to access water through the debris. We did not measure water level for this experiment but, the negative trend suggests that there is a minimum threshold for a pond's water level in regards to bat activity.

There is enough research on weather influences on bat activity to suggest a possible explanation for our activity trends. We observed a noticeable drop in activity at treatment ponds from the first to second survey session (Figure 2). During our second survey session, MMYSF experienced lower than average temperatures from June 10th to June 14th. Minimum temperatures within MMYSF ranged from 12˚C to 7˚C, averaging 4 ˚C less than the seasonal norm (US Department of Commerce 2020). Decreasing ambient temperature has a negative correlation to bat activity (Brooks 2009). We expected a positive trend in bat activity at both control and treatment ponds during the summer from the addition of newly volant juveniles. However, the abnormal weather effects could explain the reduction in bat activity during our second acoustic survey.

The south-central region of Indiana received ~76 mm to 127 mm more than the annual average rainfall in June and July 2019, (US Department of Commerce 2020), and a majority of this precipitation occurred during a three-week period that coincided with our second and third acoustic surveys. We observed a coincident decrease in bat activity at six of the 10 ponds (Figure 2 and 3) that likely stemmed from echolocation interference and suppressed insect activity during heavy rainfall (Parsons et al. 2003 and Kunz 1973). Additionally, the high volume of precipitation caused the rivers and ephemeral streams in and around MMYSF to flood bottomland habitat and agricultural fields. We did not quantify the available water outside of MMYSF, but access to drinking water is normally a constraint for bats living within the forest.

However, bottomland habitat and agricultural fields directly outside of MMYSF remained flooded throughout the rest of our 2019 survey season and, combined with flowing ephemeral streams, likely created new foraging and drinking sites that released the constraints for bats to remain within MMYSF for available drinking water and foraging sites. Bats leaving the boundaries of MMYSF could also explain why we did not see the expected increase in bat activity at pond sites from newly volant juveniles.

A possible contributing factor for bat activity not increasing at ponds after vegetation removal was that bats in our study area were unaware of the changes. Multiple bat species have shown a strong fidelity to roost and foraging sites, and return to the same locations every season (Gumbert et al. 2002; Perry 2011). Bats returning to MMYSF are likely aware of the most successful foraging locations in spatial relationship to their roosting sites. This is especially important information for pregnant and lactating bats that are more at risk of dehydration and need to reduce time spent away from their pups (Nelson and Gillam 2016). Repeat annual surveys would be required to confirm if bats have located these altered ponds, and how activity levels have changed compared to ponds with intact surrounding vegetation. Time constraints limited this experiment to just one year.

The complete picture of what drives bat use of water sources within MMYSF has more variables than we were able to test within this study. Chapter 2 showed that coverage of the surrounding vegetation structure within the midstory and canopy is an important influence on bat activity; however, other variables such as emergent vegetation coverage and surface area are also important. For this reason, management decisions based on our experiment findings should be tempered until more information is available to address this question.

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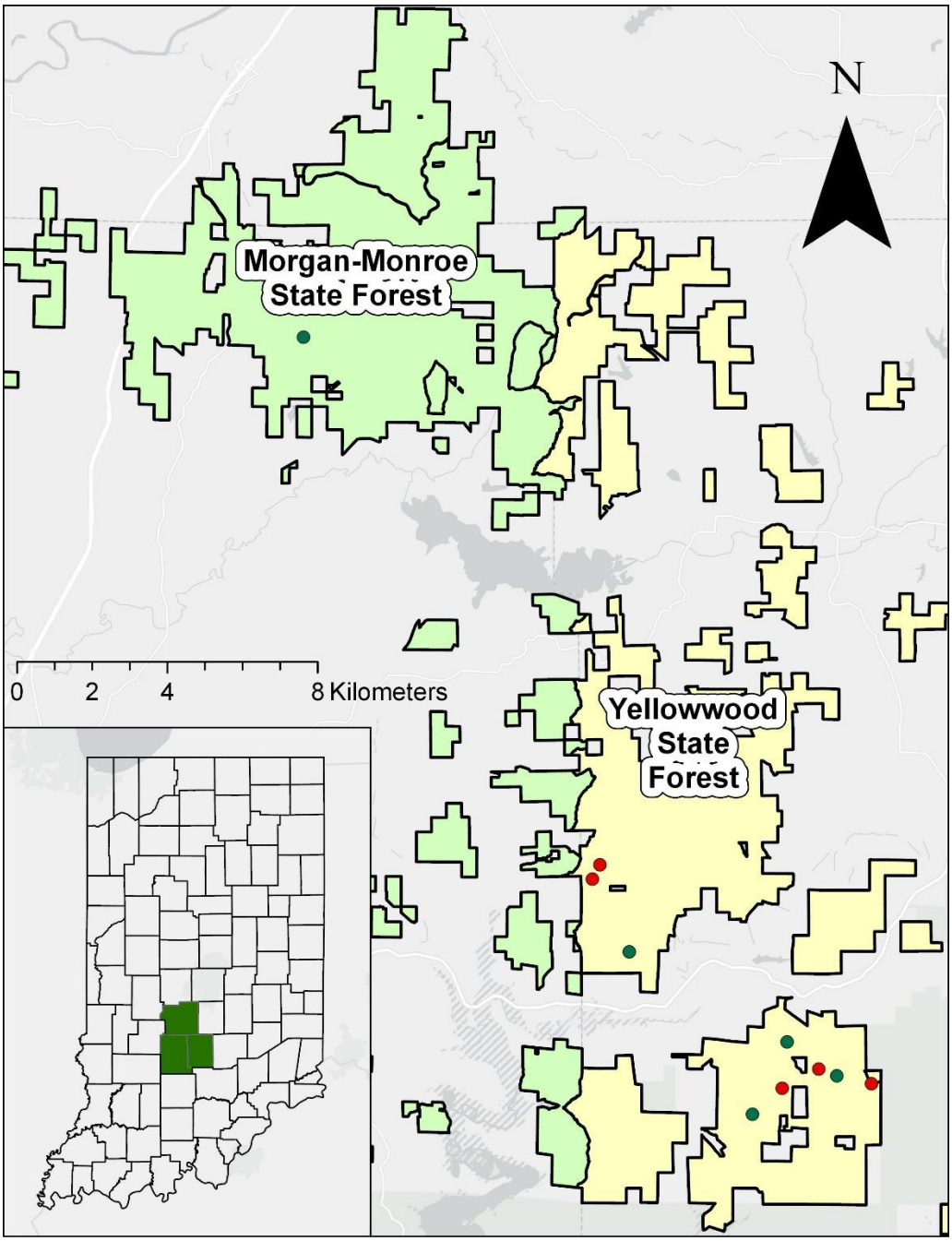

Figure 1. Map of the study area within the boundaries of the Morgan-Monroe and Yellowwood State Forests complex located in south-central Indiana, USA, May-July, 2019. Acoustic surveys were conducted at treatment ponds (green circles, n = 5) with surrounding vegetation removed and control ponds (red circles, n = 5).

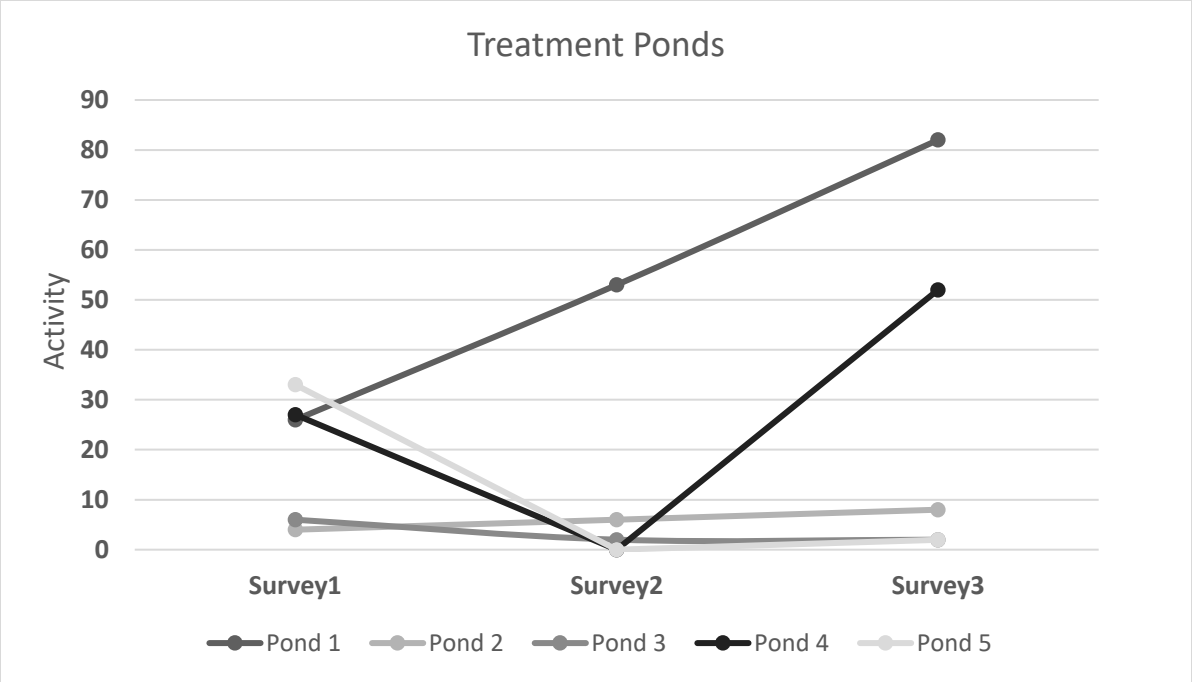

Figure 2. Results from three acoustic survey sessions at treatment ponds in 2019 from May 21st to July 22nd within MMYSF in southern Indiana. The number of bat passes for each survey were averaged and divided by survey effort (9.2 hours) to give average activity per hour. We used our 2018 acoustic survey season data to randomly select Ponds 2-4 from six ponds with the lowest activity for this experiment. Pond 1 and 5 were randomly selected from MMYSF ponds that were not surveyed in 2018.

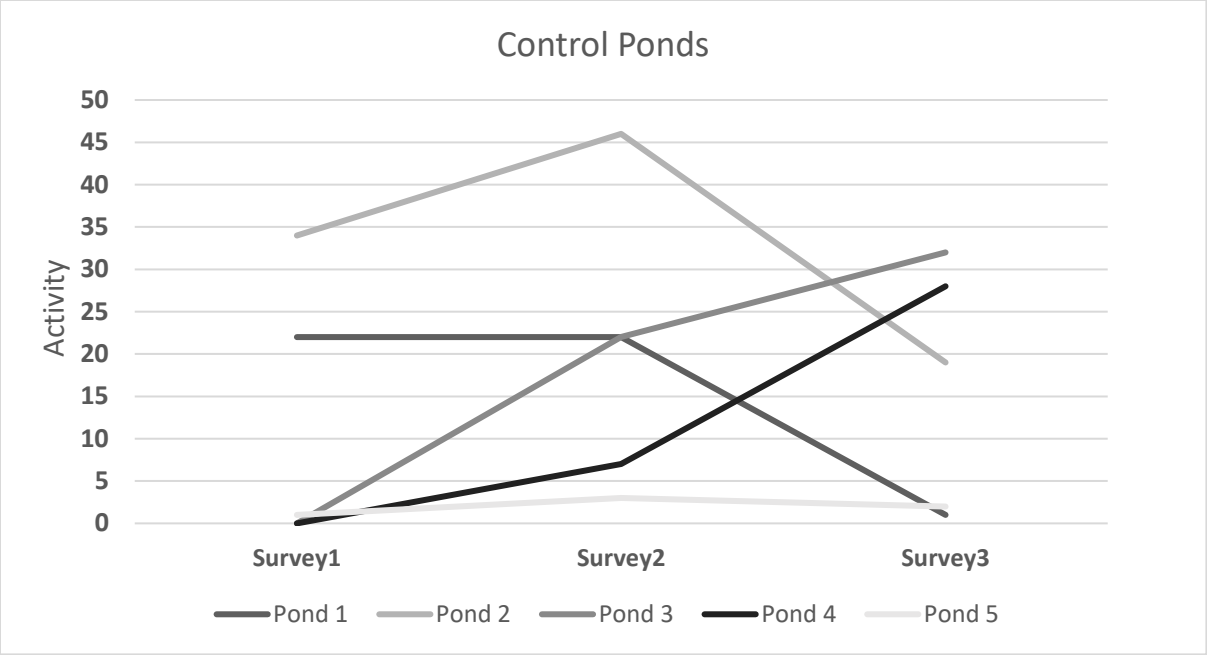

Figure 3. Results from three acoustic survey sessions at control ponds from May 21st to July 22nd within MMYSF in southern Indiana. The number of bat passes for each survey were averaged and divided by survey effort (9.2 hours) to give average activity per hour. We used our 2018 acoustic survey season data to randomly select Ponds 3-5 from six ponds with the lowest activity for this experiment. Ponds 1 and 2 were randomly selected from the remaining ponds surveyed in 2018.